

RESEARCH MONOGRAPH . COMPOUND PROFILE

# Testagen

*A synthetic tetrapeptide (Lys-Glu-Asp-Gly) developed at the Saint Petersburg Institute of Bioregulation and Gerontology as the defined active sequence of the testicular polypeptide complex Testoluten, proposed to modulate steroidogenic gene expression and Leydig cell function through direct peptide-DNA interaction and chromatin remodeling in testicular endocrine tissue.*

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## ABSTRACT

Testagen, the synthetic tetrapeptide Lys-Glu-Asp-Gly (one-letter code KEDG; molecular formula C<sub>17</sub>H<sub>29</sub>N<sub>5</sub>O<sub>9</sub>; molecular weight 447.44), is a testicular-derived bioregulatory peptide synthesized and characterized by Vladimir Khavinson and colleagues at the Saint Petersburg Institute of Bioregulation and Gerontology (IBG) beginning in the early 2000s. The compound belongs to the Khavinson class of ultrashort (two to seven residue) peptide bioregulators, a family of synthetic sequences modeled on tissue-specific peptide fragments isolated from organ extracts by acid-pepsin hydrolysis and ultrafiltration, and is the defined synthetic analog of the active component of the testicular polypeptide complex preparation Testoluten [1, 2]. Testagen is distinguished within the Khavinson bioregulator family by a testicular tissue-directed pharmacological profile that centers on three proposed activities: first, modulation of steroidogenic gene expression in Leydig cells, with reported effects on expression of genes encoding steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (CYP11A1), 3-beta-hydroxysteroid dehydrogenase (3-beta-HSD), and 17-beta-hydroxysteroid dehydrogenase, the principal rate-limiting enzymes in the testosterone biosynthetic cascade from cholesterol to testosterone [3, 4]; second, epigenetic modulation through direct peptide-DNA interaction and chromatin remodeling, consistent with the broader Khavinson hypothesis that ultrashort peptides act as sequence-specific regulators of gene expression through complementary electrostatic interactions with DNA in gene promoter regions [5, 6]; and third, normalization of hypothalamic-pituitary-gonadal (HPG) axis signaling in aged animal models,

with reported restoration of luteinizing hormone receptor expression on Leydig cells and normalization of the testosterone-to-luteinizing hormone ratio [7, 8]. The compound shares the general Khavinson bioregulator mechanism of cell and nuclear membrane penetration, direct interaction with histone proteins and double-stranded DNA, and modulation of gene transcription, a framework supported by fluorescence microscopy tracking of labeled peptide analogs into cell nuclei and by electrophoretic mobility shift assays demonstrating peptide-DNA complex formation [5, 6]. Structurally, Testagen differs from the closely related Khavinson bioregulators Epithalon (Ala-Glu-Asp-Gly, pineal-derived), Livagen (Lys-Glu-Asp-Ala, liver-derived), and Cortagen (Ala-Glu-Asp-Pro, brain-derived) by single residue substitutions that are proposed to confer tissue-specificity through differential DNA sequence recognition [9]. The substitution of lysine at position 1 (versus alanine in Epithalon) and glycine at position 4 (versus alanine in Livagen) produces a distinct charge distribution and hydrogen-bonding pattern that molecular modeling studies have associated with preferential interaction with promoter sequences of testicular steroidogenic genes [5, 10]. No formal pharmacokinetic studies have been published for Testagen. The compound, as a linear tetrapeptide with unprotected termini, is expected to undergo rapid proteolytic degradation by aminopeptidases and carboxypeptidases in plasma and gastrointestinal fluid, with a predicted plasma half-life on the order of minutes. No human clinical trials of the synthetic KEDG tetrapeptide have been registered on ClinicalTrials.gov or on major international trial registries. The clinical evidence base is limited to a single uncontrolled Russian-language clinical study and to ex vivo human cell studies. The parent polypeptide complex Testoluten has been used in Russian gerontological clinical

practice for age-related testosterone decline and male subfertility, but the synthetic tetrapeptide does not hold separate pharmaceutical registration in any jurisdiction. Testagen is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any major Western regulatory authority. It is supplied as a research-grade lyophilized powder by multiple peptide suppliers at greater than 95 percent purity by high-performance liquid chromatography. This monograph reviews the chemistry, synthesis, and structural characterization of Testagen; the proposed epigenetic, steroidogenic, and HPG axis mechanisms in molecular detail; the available pharmacokinetic considerations; the preclinical pharmacology across testicular aging and reproductive models; the limited clinical evidence; sourcing and quality verification; reconstitution and handling; stack-interaction considerations; adverse-event and safety signal; and a comparative assessment of five male reproductive or endocrine candidates against Testagen on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation).

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**Proposed International Nonproprietary Name:** None assigned. The compound is identified in the research literature exclusively as Testagen or as the tetrapeptide KEDG.

**Research names:** Testagen. The name derives from the Latin root for testis combined with a suffix convention used across the Khavinson bioregulatory peptide series (compare Vilon, Epithalon, Livagen, Cortagen, Prostamax, Ovagen, Pancragen, Bronchogen, Cardiogen, Crystagen).

**One-letter amino acid code:** KEDG.

**Three-letter amino acid code:** Lys-Glu-Asp-Gly.

**Chemical Abstracts Service registry number:** No CAS registry number has been assigned to the synthetic KEDG tetrapeptide as of the date of monograph preparation. The compound is cataloged by vendor-specific identifiers across peptide suppliers.

**PubChem Compound Identifier:** Not independently registered. The peptide sequence Lys-Glu-Asp-Gly can be retrieved through PubChem peptide sequence searches.

**International Union of Pure and Applied Chemistry name:** (S)-2-((S)-2-((S)-2-((S)-2,6-diaminohexanamido)-4-carboxybutanamido)-3-carboxypropanamido)acetic acid.

**Parent preparation:** Testagen is the defined synthetic analog of the principal active tetrapeptide isolated from Testoluten, a testicular polypeptide complex prepared by acid-pepsin hydrolysis and ultrafiltration of bovine testicular tissue [1, 2]. Testoluten contains a heterogeneous mixture of short peptides derived from both Leydig cell and Sertoli cell compartments; Testagen (KEDG) was identified as the minimal synthetic sequence that recapitulated the testicular bioregulatory activities of the parent complex. The identification of Testagen from the Testoluten extract followed the standard Khavinson methodology: acid-pepsin hydrolysis of the

tissue homogenate, ultrafiltration to isolate peptides below 10 kilodaltons, sequential chromatographic fractionation, amino acid sequencing of the bioactive fraction, and synthesis of the candidate tetrapeptide for confirmatory bioassay [2, 11].

## 1.2 Structural class and chemistry

Testagen is a linear tetrapeptide of the L-amino acid series with no cyclic, disulfide, or post-translational modifications. The structural class places it within the Khavinson ultrashort bioregulatory peptide family, a group of synthetic di- to heptapeptides modeled on tissue-specific sequences isolated from organ extracts by the acid-pepsin hydrolysis and ultrafiltration methodology developed at the Saint Petersburg Institute of Bioregulation and Gerontology [2, 9]. Other members of this family include Vilon (KE, dipeptide, thymus-derived), Epithalon (AEDG, tetrapeptide, pineal-derived), Livagen (KEDA, tetrapeptide, liver-derived), Cortagen (AEDP, tetrapeptide, brain-derived), Prostamax (KE-based, prostate-derived), Pancragen (KEDW, tetrapeptide, pancreas-derived), Bronchogen (AEDL, tetrapeptide, bronchus-derived), Cardiogen (AEDP, tetrapeptide, cardiac-derived), and Crystagen (EDP, tripeptide, immune-derived) [4, 9].

**Molecular formula:** C<sub>17</sub>H<sub>29</sub>N<sub>5</sub>O<sub>9</sub> (zwitterionic free form).

**Exact mass:** 447.1965 g/mol (monoisotopic).

**Molecular weight:** 447.44 g/mol.

**Isoelectric point:** Approximately 3.9 to 4.2, reflecting the preponderance of acidic residues (glutamic acid, aspartic acid) over the single basic residue (lysine), with glycine contributing no ionizable side chain.

**Net charge at physiological pH (7.4):** Approximately minus one. The lysine epsilon-amino group (pK<sub>a</sub> approximately 10.5) is protonated and positively charged; the glutamic acid gamma-carboxyl (pK<sub>a</sub> approximately 4.1) and the aspartic acid beta-carboxyl (pK<sub>a</sub> approximately 3.9) are deprotonated and negatively charged; the C-terminal glycine carboxyl (pK<sub>a</sub> approximately 2.3) is deprotonated and negatively charged; the N-terminal alpha-amino group (pK<sub>a</sub> approximately 8.0) is partially protonated at physiological pH.

**Physical properties:** White to off-white lyophilized powder or crystalline solid. Freely soluble in water and in aqueous buffers at physiological pH. Soluble in dimethyl sulfoxide. Sparingly soluble in apolar organic solvents. No complex ter-

tiary structure is required for activity; the peptide is conformationally flexible in solution with no stable secondary structure elements predicted by molecular dynamics simulation for a four-residue linear chain.

**Stereochemistry:** Three of the four amino acid residues (lysine, glutamic acid, aspartic acid) are in the L-configuration and contain stereocenters at the alpha-carbon. Glycine is achiral. The compound contains three stereocenters. Investigators acquiring research-grade Testagen should confirm stereochemical integrity by chiral chromatography or by comparison of optical rotation to a reference standard, as racemization at the aspartyl residue is a recognized degradation pathway for aspartate-containing peptides under acidic or prolonged storage conditions [12].

## 1.3 Synthesis

Testagen is synthesized by standard solid-phase peptide synthesis (SPPS) using fluorenylmethyloxycarbonyl (Fmoc) chemistry on a resin-bound C-terminal glycine [13]. The synthesis proceeds from C-terminus to N-terminus: the Fmoc-Gly-OH residue is coupled to a Wang resin (for the free acid C-terminus); sequential deprotection and coupling of Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, and Fmoc-Lys(Boc)-OH completes the tetrapeptide chain. Coupling reagents are typically HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate) or HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) with N,N-diisopropylethylamine as base in N,N-dimethylformamide. Cleavage from resin and simultaneous side-chain deprotection is achieved with a trifluoroacetic acid/triisopropylsilane/water cocktail (typically 95:2.5:2.5 v/v/v). The crude peptide is purified by preparative reverse-phase high-performance liquid chromatography on a C18 column with acetonitrile/water/trifluoroacetic acid gradient. Identity is confirmed by electrospray ionization mass spectrometry (expected  $[M+H]^+ = 448.20$ ) and by amino acid analysis. Purity of greater than 95 percent by analytical HPLC is standard for research-grade material; greater than 98 percent is achievable with additional chromatographic purification. The compound is lyophilized from dilute acetic acid or from water and stored as a dry powder. The synthesis is straightforward and high-yielding for a tetrapeptide; the sequence contains no sterically hindered couplings, no aggregation-prone stretches, and no unusual amino acids, and industrial-scale production presents no unusual challenges.

The notable structural relationship to Epithalon (AEDG) is that the two tetrapeptides differ only at position 1 (lysine in Testagen versus alanine in Epithalon). The substitution of the basic, long-chain lysine for the small, hydrophobic alanine introduces a positive charge and an extended side chain that are proposed to alter the DNA-binding specificity and tissue tropism of the peptide [5, 9, 10].

## 2. Abstract

Testagen (KEDG) is a synthetic tetrapeptide bioregulator developed by Vladimir Khavinson and colleagues at the Saint Petersburg Institute of Bioregulation and Gerontology as the defined active peptide of the testicular polypeptide complex Testoluten [1, 2]. The compound is proposed to modulate testicular endocrine function through epigenetic mechanisms, specifically through direct interaction of the peptide with DNA in the promoter regions of steroidogenic genes in Leydig cells, resulting in transcriptional modulation of the enzymes responsible for testosterone biosynthesis from cholesterol [3, 4, 5].

The principal proposed application is the normalization of age-related testosterone decline. In the Khavinson research framework, testicular aging is characterized by progressive heterochromatinization of euchromatic gene regions in Leydig cells, resulting in transcriptional silencing of steroidogenic enzyme genes and reduced testosterone output [7, 8]. Testagen is proposed to reverse this age-related epigenetic silencing by interacting with DNA in a sequence-specific manner, restoring transcriptional access to the silenced loci and normalizing steroidogenic enzyme expression. This mechanism has been characterized in detail for the structurally related Khavinson bioregulators Livagen and Epithalon in hepatocyte and lymphocyte models respectively [14, 15, 16], and is extrapolated to Testagen on the basis of shared structural features and the tissue-specificity principle of the Khavinson bioregulatory peptide class.

Preclinical evidence for Testagen is derived principally from the parent preparation Testoluten, which has been studied in aged male rat models and in ex vivo testicular tissue cultures. Testoluten administration to aged male rats produced normalization of testosterone levels, restoration of luteinizing hormone receptor density on Leydig cells, and histological improvement in seminiferous tubule architecture [7, 8]. The synthetic tetrapeptide KEDG has been confirmed to recapitulate these activities in cell culture models of Leydig cell function [3, 4]. Sertoli cell gene expression, including expression of androgen-binding protein and inhibin subunits, has also been reported to be modulated by Testagen in vitro, consistent with broader testicular tissue-directed activity beyond the Leydig cell compartment [4].

No formal pharmacokinetic studies have been published for Testagen. No human clinical trials meeting Western regulatory standards have been conducted. The compound does not hold pharmaceutical registration in any jurisdiction. The evidence base should be interpreted with the recognition that the published literature originates predominantly from a single research group and has not been independently replicated in Western laboratories. Testagen is supplied as a research-grade lyophilized peptide by multiple suppliers at greater than 95 percent purity. This monograph documents the chemistry, mechanism, pharmacokinetics, preclinical evidence, limited clinical evidence, sourcing, handling, interactions, safety, and comparative assessment of the compound.

## 3. Discovery and Development History

### 3.1 Origin of the Khavinson bioregulatory peptide program

The peptide bioregulator research program was initiated by Vladimir Khavinson at the Kirov Military Medical Academy in Leningrad (now Saint Petersburg) in the early 1970s, originally within the context of military gerontology and radiation protection [2, 17]. The founding observation was that polypeptide extracts prepared from specific organs (thymus, pineal gland, brain cortex, liver, prostate, testes) by acid-pepsin hydrolysis and ultrafiltration could restore organ function in aged or radiation-damaged animals in an organ-specific manner. The thymic extract preparation Thymalin (later commercialized for immunodeficiency states in Russian clinical practice) and the pineal extract Epithalamin (which produced the tetrapeptide Epithalon) were the early products of this program [2, 17, 18].

### 3.2 Isolation of the testicular peptide complex

The testicular polypeptide complex Testoluten was developed as part of the systematic extension of the Khavinson bioregulator program to the male reproductive system [1, 7]. The preparation was produced by acid-pepsin hydrolysis of bovine testicular tissue, followed by ultrafiltration to isolate peptides below 10 kilodaltons, and sequential chromatographic fractionation to enrich for the bioactive peptide fraction. Testoluten was characterized in aged male rat models beginning in the late 1990s, with the principal endpoints being serum testosterone levels, Leydig cell histology, seminiferous tubule morphology, and spermatogenesis markers [7, 8]. The preparation demonstrated restoration of testosterone production, normalization of Leydig cell ultrastructure, and improvement in spermatogenesis indices in aged animals.

### 3.3 Identification and synthesis of the active tetrapeptide

The identification of the KEDG tetrapeptide as the minimal active sequence within Testoluten followed the standard Khavinson approach: bioactivity-guided fractionation of the polypeptide complex, amino acid sequencing of the active fraction by Edman degradation, synthesis of the candidate tetrapeptide by solid-phase peptide

synthesis, and confirmatory bioassay demonstrating that the synthetic tetrapeptide recapitulated the testicular bioregulatory activities of the parent complex [2, 3]. The synthetic tetrapeptide was designated Testagen, following the naming convention of the Khavinson bioregulator series. The compound was described in Russian-language publications and patents from the Khavinson laboratory beginning in the early 2000s [3, 4, 11].

### 3.4 Relationship to other Khavinson bioregulators

Testagen occupies a specific position within the Khavinson bioregulator family defined by its tissue-of-origin (testicular) and its proposed tissue tropism (Leydig cells and Sertoli cells). The family of related tetrapeptides includes Epithalon (AEDG, pineal, telomerase activation), Livagen (KEDA, liver, hepatoprotection and chromatin decondensation), Cortagen (AEDP, brain cortex, neuroprotection), Pancragen (KEDW, pancreas, beta-cell function), Bronchogen (AEDL, bronchus, pulmonary epithelium), and Cardiogen (AEDP, heart, cardiomyocyte protection) [4, 9]. The single-residue substitutions that distinguish these tetrapeptides from one another are proposed to determine tissue specificity through differential DNA sequence recognition in organ-specific gene promoters [5, 9, 10]. This tissue-specificity principle, while experimentally supported by differential activity profiles across organ systems, has not been validated by high-resolution structural studies of peptide-DNA complexes (X-ray crystallography or cryo-electron microscopy).

### 3.5 Current development status

Testagen is not in active commercial pharmaceutical development. The parent preparation Testoluten is marketed in Russia and in some Commonwealth of Independent States jurisdictions as a dietary supplement and bioregulatory preparation for male reproductive health and age-related testosterone decline [1]. The synthetic tetrapeptide KEDG is supplied as a research-grade material by Western peptide suppliers. No investigator-initiated or sponsor-initiated clinical trials of the synthetic KEDG tetrapeptide are registered on ClinicalTrials.gov, the European Union Clinical Trials Register, or the World Health Organization International Clinical Trials Registry Platform. A ClinicalTrials.gov entry (NCT02733133) references a product called "Testagen TDS-Testosterone" that is a testosterone transdermal delivery system and is unrelated to the Khavinson bioregulatory peptide [19].

## 4. Mechanism of Action

Testagen is proposed to act through the general Khavinson bioregulatory peptide mechanism of direct peptide-DNA interaction and epigenetic modulation of gene expression, with tissue specificity conferred by the KEDG amino acid sequence and its preferential interaction with promoter regions of testicular steroidogenic genes [3, 4, 5, 6].

### 4.1 General Khavinson bioregulatory peptide mechanism

The Khavinson hypothesis proposes that ultrashort peptides (two to seven residues) can traverse the plasma membrane, the cytoplasm, and the nuclear membrane to reach the nucleus and nucleolus of target cells, where they interact directly with histone proteins and with double-stranded DNA to modulate chromatin structure and gene transcription [5, 6]. The mechanism has been supported by the following experimental observations across the Khavinson bioregulator family:

First, fluorescence microscopy tracking of labeled Khavinson peptides (including the dipeptide KE and the tetrapeptides AEDG and KEDA) has demonstrated intracellular localization in the cytoplasm, nucleus, and nucleolus of cultured human cells within minutes of extracellular application [5, 6, 20]. The mechanism of membrane transit is proposed to involve passive diffusion facilitated by the small size and the amphipathic charge distribution of the peptides, though carrier-mediated transport through peptide transporters (PEPT1, PEPT2) has also been proposed for oral absorption [21].

Second, electrophoretic mobility shift assays (EMSA) have demonstrated formation of peptide-DNA complexes between Khavinson tetrapeptides and double-stranded DNA oligonucleotides, with sequence specificity: the tetrapeptide KEDW (Pancragen) showed preferential binding to the sequence ACCT in pancreatic gene promoters, and the dipeptide KE (Vilon) showed binding to the sequence TCGA in the APG5L gene promoter [5, 6]. The DNA-binding specificity of KEDG (Testagen) has not been independently characterized by EMSA or by other biophysical methods, but is predicted by molecular modeling to favor promoter sequences enriched in testicular steroidogenic genes.

Third, chromatin immunoprecipitation (ChIP) assays with the tetrapeptide KEDA (Livagen) demonstrated enhanced acetylation of histone H3 at promoter regions of specific liver genes in hepatocyte cultures from aged donors, consistent with peptide-induced chromatin decondensation and transcriptional activation [14, 15]. The related tetrapeptide AEDG (Epithalon) was shown by a comparative study of five Khavinson peptides to induce deheterochromatinization of pericentromeric structural heterochromatin of chromosomes 1 and 9 in lymphocytes from donors aged 75 to 88 years [16].

## 4.2 Testicular tissue-specific mechanism

The proposed testicular tissue-specific mechanism of Testagen centers on modulation of gene expression in the two principal cell types of the testicular interstitium and seminiferous epithelium: Leydig cells and Sertoli cells [3, 4].

In Leydig cells, the proposed primary targets are the genes encoding the key enzymes and regulatory proteins of the steroidogenic cascade:

- **StAR (steroidogenic acute regulatory protein):** The rate-limiting step in testosterone biosynthesis is the transport of cholesterol from the outer to the inner mitochondrial membrane, mediated by StAR. Testagen is proposed to upregulate StAR gene expression in aged Leydig cells, restoring cholesterol transport and initiating the steroidogenic cascade [3, 4, 22].
- **CYP11A1 (cholesterol side-chain cleavage enzyme, P450<sub>scc</sub>):** The first enzymatic step in steroidogenesis, the conversion of cholesterol to pregnenolone in the mitochondrial inner membrane. CYP11A1 expression declines with Leydig cell aging and is proposed to be restored by Testagen-mediated transcriptional activation [3, 23].
- **3-beta-HSD (3-beta-hydroxysteroid dehydrogenase):** Catalyzes the conversion of pregnenolone to progesterone and of dehydroepiandrosterone to androstenedione. Expression of the 3-beta-HSD type 2 isoform (the principal testicular form) is proposed to be modulated by Testagen [3, 4].
- **CYP17A1 (17-alpha-hydroxylase/17,20-lyase):** Catalyzes the 17-alpha-hydroxylation of pregnenolone and progesterone and the subsequent cleavage to dehydroepiandrosterone and androstenedione. This enzyme activity defines the delta-4 and delta-5 steroidogenic pathways in the testis [23].

- **17-beta-HSD (17-beta-hydroxysteroid dehydrogenase):** The terminal enzyme in testosterone biosynthesis, catalyzing the reduction of androstenedione to testosterone. The type 3 isoform (17-beta-HSD3) is the principal testicular form [23].

In Sertoli cells, Testagen is proposed to modulate expression of androgen-binding protein (which concentrates testosterone in the seminiferous tubule lumen for spermatogenesis), inhibin B subunits (which provide negative feedback to the pituitary and are markers of Sertoli cell function), and transferrin (which supports iron delivery to developing germ cells) [4, 24].

### 4.3 HPG axis modulation

The hypothalamic-pituitary-gonadal (HPG) axis regulates testicular testosterone production through the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which drives pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion, which in turn stimulate Leydig cell testosterone production and Sertoli cell spermatogenesis support respectively [25]. Testagen is proposed to normalize HPG axis function in aged animals through a peripheral (testicular) mechanism rather than a central (hypothalamic or pituitary) mechanism: by restoring Leydig cell sensitivity to LH through upregulation of LH receptor expression, the peptide is proposed to restore testosterone production at the existing level of LH stimulation, rather than by increasing LH secretion [7, 8]. This peripheral mechanism distinguishes the proposed Testagen activity from the central mechanisms of GnRH analogs (gonadorelin, leuprolide) and kisspeptin, which act upstream at the hypothalamic-pituitary level.

Studies of the parent preparation Testoluten in aged male rats reported normalization of the testosterone-to-LH ratio (an index of Leydig cell LH sensitivity) and increased LH receptor density on Leydig cells by immunohistochemistry [7, 8]. Whether the synthetic KEDG tetrapeptide produces identical HPG axis normalization has not been independently demonstrated in published studies.

## 4.4 Epigenetic specificity and the tissue-specificity question

The central claim of the Khavinson bioregulator framework is that single-residue substitutions among structurally related short peptides produce organ-specific biological effects through differential DNA sequence recognition [5, 9, 10]. The evidence for this claim includes the differential biological activity profiles observed across the bioregulator family (Epithalon on pinealocytes, Livagen on hepatocytes, Testagen on Leydig cells, Pancragen on pancreatic beta cells, and so on) and molecular modeling studies predicting preferential peptide-DNA binding at different promoter sequences [5, 10].

The limitations of this evidence should be noted. No high-resolution structural data (X-ray crystallography, cryo-electron microscopy, or nuclear magnetic resonance) for any Khavinson tetrapeptide-DNA complex have been published. The molecular modeling studies employ computational docking approaches that predict, but do not confirm, sequence-specific binding. The electrophoretic mobility shift assay data demonstrating peptide-DNA complex formation have been reported for a limited number of tetrapeptides and DNA sequences, and the binding affinities (which would distinguish specific from nonspecific electrostatic interaction) have not been quantified in most published reports. The biological activity differences between structurally related tetrapeptides could reflect differential cellular uptake, differential intracellular localization, differential proteolytic stability, or differential interaction with proteins other than DNA, rather than direct DNA sequence specificity.

# 5. Pharmacokinetics and Routes of Administration

## 5.1 General considerations

No formal pharmacokinetic studies of the synthetic KEDG tetrapeptide have been published in the peer-reviewed literature. The pharmacokinetic profile of Testagen is inferred from general principles of tetrapeptide pharmacokinetics and from limited data on structurally related Khavinson bioregulators [21, 26].

## 5.2 Absorption

Testagen, as a linear tetrapeptide of molecular weight 447.44, is within the size range accessible to intestinal peptide transporters (PEPT1, SLC15A1), which transport di- and tripeptides and some tetrapeptides across the intestinal epithelium by proton-coupled cotransport [21]. Whether KEDG is a substrate for PEPT1 has not been experimentally determined. Oral bioavailability of linear tetrapeptides is generally low (typically less than 5 percent) owing to extensive luminal degradation by gastric and pancreatic proteases (pepsin, trypsin, chymotrypsin) and brush-border peptidases (aminopeptidases, carboxypeptidases, dipeptidyl peptidases) [26]. The Khavinson laboratory has published molecular modeling studies predicting that certain ultrashort peptides, including sequences related to KEDG, interact with the peptide transporter PEPT1 and the amino acid transporter LAT1, potentially facilitating intestinal absorption [21]. The practical oral bioavailability of Testagen in humans remains undetermined.

Subcutaneous administration of tetrapeptides generally provides higher systemic bioavailability than oral administration, though still subject to rapid proteolytic degradation at the injection site and in the systemic circulation. Intranasal administration has been proposed for Khavinson peptides as an alternative route that may bypass first-pass hepatic and gastrointestinal degradation [26].

## 5.3 Distribution

No distribution data are available for Testagen. By analogy with other Khavinson peptides, the compound is predicted to distribute broadly to tissues with a relatively low volume of distribution (consistent with a hydrophilic, charged peptide with limited membrane partitioning). The Khavinson laboratory has demonstrated intracellular distribution to the cytoplasm, nucleus, and nucleolus using fluorescence-labeled peptide analogs, suggesting that the peptide reaches intracellular targets despite its hydrophilic character [5, 6, 20]. The mechanism of testicular tissue localization (whether the peptide accumulates preferentially in testicular tissue or whether the tissue-specificity is conferred by differential biological activity at equivalent tissue concentrations) has not been determined.

## 5.4 Metabolism and elimination

Testagen is expected to be metabolized by ubiquitous proteolytic enzymes (aminopeptidases, carboxypeptidases, and endopeptidases) in plasma, liver, kidney, and other tissues, producing the constituent amino acids (lysine, glutamic acid, aspartic acid, glycine), which enter normal amino acid metabolic pools [26]. The predicted plasma half-life is on the order of minutes, consistent with published half-lives for unprotected linear tetra- and pentapeptides in mammalian plasma [26]. No accumulation is expected with repeated dosing at standard intervals.

Renal excretion of the intact peptide is predicted to be negligible, as the peptide is expected to be fully degraded to amino acids before reaching the renal tubule at significant concentrations.

## 5.5 Routes of administration

The principal routes used in Khavinson bioregulator research and practice are:

- **Subcutaneous injection:** The most common route for research-grade synthetic bioregulators. Typical research protocols describe reconstitution in bacteriostatic water for injection and subcutaneous administration [1, 4].
- **Oral (capsule):** The parent preparation Testoluten is marketed as an oral capsule preparation in Russian clinical practice, with typical dosing of 1 to 2 capsules (each containing the polypeptide complex) daily for 10 to 30 days [1, 7].

The synthetic KEDG tetrapeptide is also described in oral dosing protocols in the research literature, though the oral bioavailability limitation noted above applies.

- **Intranasal:** Proposed but not extensively characterized. May offer advantages for bypassing gastrointestinal degradation.

## 5.6 Special populations

No data are available for any special population (pediatric, geriatric, hepatic impairment, renal impairment, pregnancy, lactation). The compound is intended for research use in adult populations.

# 6. Preclinical Pharmacology

## 6.1 Testoluten studies in aged male rats

The principal preclinical evidence base for the testicular bioregulatory activity of the Testagen lineage derives from studies of the parent polypeptide complex Testoluten in aged male rats [7, 8]. These studies, conducted at the Saint Petersburg Institute of Bioregulation and Gerontology, used rats aged 18 to 24 months (corresponding to advanced reproductive senescence) and characterized the following endpoints:

**Testosterone levels:** Aged male rats treated with Testoluten for 10 to 30 days showed significant increases in serum testosterone compared to age-matched untreated controls, with treated values approaching those of young (3 to 4 month) animals [7, 8]. The magnitude of testosterone restoration was typically 40 to 70 percent of the age-related deficit.

**Leydig cell histology and ultrastructure:** Electron microscopy of testicular sections from Testoluten-treated aged rats showed increased smooth endoplasmic reticulum (the site of steroidogenic enzyme activity), normalization of mitochondrial morphology (with increased cristae density, consistent with enhanced CYP11A1 activity), and restoration of lipid droplet content (the cholesterol substrate reservoir for steroidogenesis) compared to untreated aged controls [7, 8].

**LH receptor density:** Immunohistochemical staining for LH receptor on Leydig cells demonstrated increased receptor density in Testoluten-treated aged rats compared to age-matched controls, consistent with the proposed mechanism of restored LH sensitivity [7, 8].

**Seminiferous tubule morphology:** Testoluten-treated aged rats showed improvement in seminiferous tubule architecture, with increased germinal epithelium height, reduced vacuolization, and increased spermatogenesis activity as assessed by spermatogonial cell counts and spermatid maturation indices [7, 8].

These studies are limited by the use of the polypeptide complex rather than the purified synthetic tetrapeptide, by the absence of dose-response characterization, by the small sample sizes typical of Russian gerontological studies, and by the ab-

sence of independent replication.

## 6.2 Synthetic KEDG tetrapeptide studies in cell culture

Cell culture studies of the synthetic KEDG tetrapeptide have been reported in Leydig cell and Sertoli cell models [3, 4]:

**Leydig cell models:** Treatment of primary rat Leydig cells and of the MA-10 mouse Leydig tumor cell line with Testagen at concentrations of 10 to 100 nanograms per milliliter for 24 to 72 hours produced increases in StAR mRNA expression (1.5- to 2.5-fold), CYP11A1 mRNA expression (1.3- to 1.8-fold), and testosterone secretion into culture medium (1.4- to 2.0-fold) compared to vehicle-treated controls [3, 4]. The dose-response relationship was described as bell-shaped, with maximal effect at 20 to 50 nanograms per milliliter and reduced effect at 200 nanograms per milliliter and above, consistent with the inverted-U dose-response curves reported for other Khavinson bioregulators [3].

**Sertoli cell models:** Treatment of primary rat Sertoli cells with Testagen at comparable concentrations produced increases in androgen-binding protein secretion (1.3- to 1.6-fold) and inhibin B subunit mRNA expression (1.2- to 1.5-fold) [4]. These effects are consistent with a broader testicular tissue-directed activity that encompasses both the steroidogenic (Leydig) and the spermatogenesis-support (Sertoli) compartments.

## 6.3 Gene expression studies

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) studies of Leydig cells treated with Testagen have characterized the steroidogenic gene expression profile [3, 4]. In addition to the StAR and CYP11A1 upregulation noted above, the following effects have been reported:

- 3-beta-HSD type 2 mRNA: 1.2- to 1.6-fold increase at 24 hours
- CYP17A1 mRNA: 1.1- to 1.4-fold increase at 24 hours
- 17-beta-HSD type 3 mRNA: 1.2- to 1.5-fold increase at 48 hours
- LH receptor mRNA: 1.3- to 1.7-fold increase at 48 hours

The gene expression changes are modest in magnitude and have not been characterized at the protein expression level (Western blot, immunohistochemistry) in published studies of the synthetic KEDG tetrapeptide specifically. Whether these

transcriptional changes translate to functionally meaningful increases in enzyme activity and testosterone output at the tissue and organismal level requires confirmation in intact animal studies with the purified synthetic peptide.

## 6.4 Chromatin remodeling studies

Direct chromatin remodeling studies of Testagen have not been published. By analogy with Livagen (KEDA) and Epithalon (AEDG), Testagen is predicted to induce chromatin decondensation (deheterochromatinization) in testicular cells from aged donors [14, 15, 16]. The Livagen chromatin data (histone H3 acetylation enhancement at liver gene promoters demonstrated by ChIP assay) and the Epithalon chromosome 1/9 pericentromeric deheterochromatinization data provide the mechanistic framework, but Testagen-specific chromatin data represent a gap in the evidence base.

## 6.5 Lifespan and aging studies

The Khavinson laboratory has published extensive lifespan data for the parent preparations (thymic, pineal, and other organ extracts) and for certain synthetic bioregulators (notably Epithalon and Vilon) demonstrating lifespan extension in rodent and *Drosophila* models [17, 18, 27]. No specific lifespan data have been published for Testagen or Testoluten.

## 7. Clinical Evidence Base

### 7.1 Testoluten clinical use in Russian gerontological practice

The parent polypeptide complex Testoluten has been used in Russian gerontological clinical practice for age-related testosterone decline, male subfertility, and recovery of reproductive function after infectious or inflammatory testicular disease [1, 7]. The preparation is marketed as a dietary supplement rather than a registered pharmaceutical in Russia. Clinical use is described in Russian-language monographs and in the Saint Petersburg Institute of Bioregulation and Gerontology publications, but the clinical evidence has not been reported in the format of Western regulatory-standard randomized controlled trials.

### 7.2 Uncontrolled clinical study

A single uncontrolled clinical study of Testoluten in men aged 40 to 65 years with biochemical evidence of age-related testosterone decline (total testosterone below 300 nanograms per deciliter) has been described in Khavinson laboratory publications [7, 8]. The study reportedly enrolled approximately 30 subjects who received Testoluten capsules daily for 30 days. Endpoints included serum total testosterone, free testosterone, luteinizing hormone, follicle-stimulating hormone, and subjective symptom scores (libido, energy, mood). The reported outcomes included statistically significant increases in total testosterone (mean increase of approximately 15 to 25 percent from baseline) and improvement in subjective symptom scores compared to baseline [7, 8].

The limitations of this study are substantial: the absence of a placebo control group, the absence of randomization, the small sample size, the absence of blinding, the absence of independent outcome adjudication, the reliance on the parent polypeptide complex rather than the purified synthetic tetrapeptide, and the absence of publication in a peer-reviewed Western journal. The study does not meet the evidentiary standard for clinical efficacy determination.

## 7.3 Ex vivo human tissue studies

Ex vivo studies of Khavinson bioregulators in human tissues have been conducted for several members of the family (Livagen in human lymphocytes from aged donors, Epithalon in human lymphocytes from aged donors, Vilon in human thymic tissue) [14, 15, 16]. Testagen-specific ex vivo human testicular tissue studies have not been reported in the accessible literature. Given the ethical and practical constraints on human testicular biopsy for research purposes, ex vivo testicular tissue studies are inherently limited in feasibility.

## 7.4 Clinical evidence gap

The clinical evidence base for Testagen is the weakest of the Khavinson bioregulators reviewed in this monograph series. Unlike Epithalon (which has published data on telomerase activation in human blood cells) and Livagen (which has published data on chromatin decondensation in human lymphocytes from aged donors), Testagen lacks any published human data from a study design that permits causal inference. The compound should be regarded as preclinical in its evidence maturity, with the parent preparation Testoluten providing the only clinical data point, and that data point falling below the standard required for efficacy determination.

## 8. Sourcing and Quality Verification

Research-grade Testagen is supplied by multiple peptide and research chemical suppliers (Peptide Sciences, BioLongevity Labs, Core Peptides, Limitless Biotech, and others) at greater than 95 percent purity by HPLC, typically as a lyophilized powder in quantities of 10 to 50 milligrams [28]. The compound is also available from Chinese and Indian peptide contract synthesis organizations at competitive pricing for larger quantities.

No pharmaceutical-grade preparation of the synthetic KEDG tetrapeptide exists. The parent polypeptide complex Testoluten is available through Russian and CIS-region suppliers as an oral capsule preparation, but the complex is not a defined single-entity product and is not suitable for research requiring a characterized synthetic compound.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 95 percent (greater than 98 percent preferred for dose-response studies)
- Mass spectrometry confirmation of molecular weight (expected  $[M+H]^+ = 448.20$  for the protonated free form)
- Amino acid analysis to confirm the Lys:Glu:Asp:Gly stoichiometry at 1:1:1:1
- Chiral amino acid analysis or chiral HPLC to confirm L-configuration of all stereocenters, particularly the aspartyl residue (which is susceptible to racemization under acidic storage conditions) [12]
- Residual solvent analysis (gas chromatography for trifluoroacetic acid, acetonitrile, and dimethylformamide, common SPPS solvents)
- Endotoxin testing (Limulus amoebocyte lysate assay) for material intended for cell culture or in vivo research

The trifluoroacetate counterion from the HPLC purification step is typically present in research-grade material. For applications where trifluoroacetate toxicity is a concern (particularly cell culture at high peptide concentrations), acetate salt exchange can be performed by lyophilization from dilute acetic acid.

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. The research-grade Testagen market is newer and smaller than markets for better-established peptides; lot-to-lot variability should be expected, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

## 9. Reconstitution and Handling

Testagen is supplied as a lyophilized powder and is reconstituted prior to use. The compound is freely soluble in water at room temperature.

**Stock solution preparation:** Reconstitute the lyophilized powder in bacteriostatic water for injection (for subcutaneous administration) or in sterile phosphate-buffered saline (for cell culture applications) at a concentration of 1 to 10 milligrams per milliliter. Vortex gently or allow to dissolve at room temperature for 5 to 10 minutes. Do not heat. The peptide dissolves readily without the need for organic cosolvents.

**DMSO stock solutions:** For in vitro applications requiring higher concentrations, the peptide can be dissolved in dimethyl sulfoxide at concentrations up to 50 milligrams per milliliter. DMSO stocks should be aliquoted to avoid freeze-thaw cycles and stored at minus 20 degrees Celsius.

**Storage of lyophilized powder:** Store at minus 20 degrees Celsius in a desiccated container. The lyophilized powder is stable for at least 24 months under these conditions. Brief excursions to room temperature (during weighing and reconstitution) do not affect potency.

**Storage of reconstituted solutions:** Aqueous solutions reconstituted with bacteriostatic water for injection should be stored at 2 to 8 degrees Celsius and used within 14 to 30 days. Aqueous solutions reconstituted with sterile water (without bacteriostatic agent) should be stored at 2 to 8 degrees Celsius and used within 7 days, or aliquoted and frozen at minus 20 degrees Celsius for longer-term storage. Frozen aqueous aliquots are stable for at least 6 months. Avoid repeated freeze-thaw cycles; aliquot into single-use volumes.

**Light sensitivity:** The compound does not contain chromophores that are sensitive to ambient light. Light protection is not required but is reasonable practice for long-term storage.

**Sterile technique:** For cell culture and in vivo applications, reconstituted solutions should be sterile-filtered through a 0.22-micrometer syringe filter before use. The small molecular weight of the tetrapeptide ensures complete passage through

the filter without adsorptive loss.

**Compatibility:** Testagen is compatible with standard laboratory plasticware (polypropylene tubes, polystyrene culture plates) and with glass. No significant adsorptive losses to polypropylene or glass surfaces are expected for a hydrophilic peptide at the concentrations used in research.

## 10. Stack Interactions and Combinations

Testagen lacks formal drug interaction studies. The following considerations are derived from the proposed mechanism of action, from the structural and functional relationships to other bioregulators and endocrine agents, and from general principles of peptide pharmacology.

**Other Khavinson bioregulators:** Concurrent administration of Testagen with other Khavinson bioregulators (Epithalon, Livagen, Vilon, Cortagen, Pancragen, Bronchogen, Cardiogen, Crystagen) is described in Russian gerontological practice as part of multi-organ "bioregulatory courses" [1, 2]. No formal interaction studies have been conducted. The Khavinson framework proposes that the tissue specificity of each bioregulator limits the potential for off-target interaction, though this has not been experimentally validated. The structural similarity of the tetrapeptides (single-residue substitutions) raises the theoretical possibility of competitive interaction at shared DNA-binding sites.

**Exogenous testosterone and testosterone precursors:** Concurrent administration of Testagen with exogenous testosterone (testosterone cypionate, testosterone enanthate, testosterone undecanoate) or with testosterone precursors (dehydroepiandrosterone, androstenedione) would be pharmacologically redundant for the testosterone-elevation endpoint and could confound the interpretation of Testagen-specific effects. Investigators should consider washout periods for exogenous androgens before initiating Testagen research protocols.

**Selective estrogen receptor modulators (SERMs) and aromatase inhibitors:** Concurrent enclomiphene, clomiphene, tamoxifen, or anastrozole with Testagen would target different nodes of the HPG axis (SERMs and aromatase inhibitors act at the hypothalamic-pituitary level to increase gonadotropin secretion, whereas Testagen is proposed to act at the testicular level to enhance Leydig cell sensitivity to existing gonadotropin stimulation). The combination is theoretically synergistic and has not been studied.

**GnRH agonists and antagonists:** GnRH agonists (leuprolide, goserelin) in chronic administration suppress gonadotropin secretion through pituitary GnRH receptor desensitization, producing chemical castration. Concurrent Testagen administration during GnRH agonist-induced suppression would be expected to be ineffective, as the Leydig cell is deprived of LH stimulation regardless of LH receptor density. GnRH antagonists (degarelix, cetrorelix) produce immediate gonadotropin suppression with the same consequence.

**Human chorionic gonadotropin (hCG):** hCG is a structural and functional analog of LH that directly stimulates Leydig cell testosterone production through the LH receptor. Concurrent administration of hCG with Testagen would be expected to produce additive effects if Testagen increases LH receptor density or steroidogenic enzyme expression. This combination has not been studied.

**Kisspeptin:** Kisspeptin peptides act at the hypothalamic level to stimulate GnRH secretion, increasing pituitary LH and FSH output. Concurrent kisspeptin and Testagen would target complementary nodes of the HPG axis (central and peripheral respectively) and might produce synergistic testosterone elevation. This combination has not been studied.

**CYP enzyme considerations:** Testagen, as a tetrapeptide metabolized by proteolytic degradation, is not expected to interact with cytochrome P450 enzymes as either a substrate, inhibitor, or inducer. No CYP-mediated drug-drug interactions are predicted.

# 11. Adverse Events and Safety Signal

## 11.1 Published safety data

No formal safety or toxicology studies of the synthetic KEDG tetrapeptide have been published in the peer-reviewed literature. The safety profile is inferred from the following sources:

**General tetrapeptide safety:** Linear tetrapeptides composed of proteinogenic L-amino acids are generally regarded as having low intrinsic toxicity, as their degradation products are the constituent amino acids (lysine, glutamic acid, aspartic acid, glycine), all of which are normal dietary components and intermediary metabolites [26]. The rapid proteolytic degradation and negligible accumulation predicted for Testagen further limit the potential for systemic toxicity.

**Khavinson bioregulator class safety:** The Khavinson laboratory has reported that bioregulatory peptides, including Epithalon, Livagen, Vilon, and Thymalin, have been used in over 15 million patient-courses in Russian clinical practice without reports of serious adverse events [2, 17]. The specific contribution of Testoluten to this safety database has not been separately quantified. These safety claims originate from a single research group and have not been independently verified through Western pharmacovigilance systems.

**Testoluten capsule safety:** The parent preparation Testoluten, marketed as an oral capsule in Russia, is reportedly well tolerated. No specific adverse events have been reported in the limited published clinical data [7, 8]. The absence of reported adverse events may reflect the genuinely low toxicity of the preparation, the small number of subjects studied, the short observation period, or the incomplete adverse-event reporting practices in uncontrolled studies.

## 11.2 Theoretical safety considerations

**Androgenic effects:** If Testagen produces clinically meaningful increases in testosterone levels, the compound would carry the class risks associated with androgen excess, including erythrocytosis (increased red blood cell production driven by testosterone stimulation of renal erythropoietin), unfavorable lipid profile changes

(reduction in high-density lipoprotein cholesterol, potential increase in low-density lipoprotein cholesterol), hepatic enzyme elevation, acne, male-pattern hair loss in genetically susceptible individuals, and prostatic effects (prostatic hypertrophy, acceleration of occult prostatic malignancy) [29, 30]. These risks are directly proportional to the magnitude and duration of testosterone elevation and are well characterized for exogenous testosterone administration. Whether the modest testosterone increases reported for Testoluten (15 to 25 percent from baseline) would produce clinically meaningful androgenic adverse effects is unknown.

**Prostatic safety:** Any compound that increases testicular testosterone production raises the question of prostatic safety. Testosterone is converted to dihydrotestosterone by 5-alpha-reductase in prostatic tissue, and dihydrotestosterone is the principal androgen driving prostatic growth. Men with occult or established prostatic malignancy should not use compounds intended to increase testosterone production without appropriate oncological evaluation [30].

**Reproductive effects:** In men with intact fertility, increased testosterone levels may paradoxically suppress spermatogenesis through negative feedback on the HPG axis (increased testosterone suppresses pituitary LH and FSH, reducing intratesticular testosterone and FSH-driven Sertoli cell function). This paradoxical fertility-suppressive effect is the basis of investigational male hormonal contraception [31]. Whether Testagen, through its proposed peripheral mechanism (Leydig cell sensitization rather than hypothalamic suppression), would avoid this paradox is unknown.

**Cardiovascular effects:** The relationship between testosterone levels and cardiovascular risk is complex and contested. Some observational studies and meta-analyses have reported increased cardiovascular event rates with testosterone replacement therapy, while others have not confirmed this association [32]. Any compound that modulates testosterone levels requires cardiovascular safety monitoring in research applications.

## 11.3 Immunogenicity

Testagen, as a tetrapeptide composed of proteinogenic amino acids, is below the typical immunogenic threshold for peptide antigens (which generally require at least 8 to 10 residues for MHC class I or II presentation) [33]. Immunogenic reactions to the peptide itself are not expected. However, contaminants in research-

grade preparations (endotoxin, residual solvents, TFA counterion) could produce non-specific inflammatory or hypersensitivity responses, particularly in in vivo applications.

## 11.4 Pregnancy and lactation

No data are available. The compound is intended for research in adult male subjects and is not indicated for use in women. Given the androgenic potential, use in pregnant or lactating women would be contraindicated by the general principle of avoiding androgenic exposures during pregnancy (risk of virilization of a female fetus) [34].

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five male reproductive or endocrine candidates against Testagen, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five candidates were selected to represent the principal pharmacological approaches to male testosterone modulation and to clarify the positioning of Testagen within the broader landscape of compounds that target the HPG axis or testicular function.

### 12.1 Gonadorelin (GnRH)

**Mechanism:** Gonadorelin is the synthetic decapeptide form of endogenous gonadotropin-releasing hormone (pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>). It stimulates pituitary gonadotroph cells to release luteinizing hormone and follicle-stimulating hormone through the GnRH type 1 receptor, producing downstream stimulation of Leydig cell testosterone production [35].

**Development trajectory:** Gonadorelin is a well-established pharmaceutical compound approved by the FDA for the evaluation of hypothalamic-pituitary function and for certain reproductive endocrine indications. It is the reference compound for GnRH receptor pharmacology.

**Novelty:** Low. The endogenous peptide hormone; no novelty as a therapeutic concept.

**Effect size:** Large. Pulsatile GnRH administration produces robust LH and testosterone elevation in hypogonadotropic hypogonadism. Continuous administration produces paradoxical suppression through receptor desensitization (the basis of GnRH agonist therapy in prostate cancer and endometriosis) [35].

**Promising potential:** Established. Approved pharmaceutical with defined clinical applications. Not a novel candidate.

**Side-effect profile:** Pulsatile administration is well tolerated. Continuous administration produces the intended chemical castration with associated hot flashes, bone density loss, metabolic syndrome, and sexual dysfunction. The pulsatile administration route (requiring subcutaneous pump delivery) limits practical applicability.

**Overall validation:** High. Extensive clinical validation across multiple indications over decades. The reference standard for HPG axis pharmacology.

**Comparison to Testagen:** Gonadorelin acts centrally at the pituitary to drive LH secretion, whereas Testagen is proposed to act peripherally at the Leydig cell to enhance LH sensitivity. The mechanisms are complementary rather than competitive. Gonadorelin has comprehensive clinical validation and FDA approval; Testagen has no clinical validation meeting Western regulatory standards. Gonadorelin has well-characterized pharmacokinetics and safety; Testagen has neither. The comparison highlights the vast evidence gap between Testagen and an established endocrine pharmaceutical.

## 12.2 Kisspeptin-10

**Mechanism:** Kisspeptin-10 is the C-terminal decapeptide fragment of kisspeptin-54, the endogenous ligand for the KISS1R receptor (GPR54) on hypothalamic GnRH neurons. Kisspeptin acts upstream of GnRH, stimulating GnRH neuronal firing and pulsatile GnRH release, thereby increasing pituitary LH and FSH secretion and downstream testosterone production [36, 37].

**Development trajectory:** Kisspeptin-10 has been studied in multiple Phase 1 and Phase 2 clinical trials for hypogonadotropic hypogonadism, hypothalamic amenorrhea, and as a diagnostic tool for HPG axis function. Clinical studies by Dhillon et al. and by the Imperial College London group have demonstrated acute and sustained gonadotropin and testosterone elevation in healthy men and in men with functional hypogonadotropic hypogonadism [36, 37].

**Novelty:** High. The kisspeptin system was discovered in 2003 as the upstream regulator of GnRH secretion, representing a fundamentally new pharmacological target in reproductive endocrinology.

**Effect size:** Moderate to large. Intravenous kisspeptin-10 administration produces rapid, dose-dependent increases in LH and testosterone in healthy men, with testosterone increases of 50 to 150 percent from baseline in acute studies [36, 37].

**Promising potential:** Moderate to high. Active clinical investigation. No approved pharmaceutical indication yet, but clinical development is progressing in reproductive endocrinology.

**Side-effect profile:** Phase 1 and Phase 2 studies have reported excellent tolerability with no serious adverse events. The theoretical concern of tachyphylaxis (desensitization of KISS1R with continuous exposure) has been observed in some protocols.

**Overall validation:** Moderate. Multiple clinical studies with consistent results. No approved indication. The compound is earlier in clinical development than gonadorelin at the time of its introduction but has a strong translational trajectory.

**Comparison to Testagen:** Kisspeptin-10 acts at the most upstream node of the HPG axis (hypothalamic kisspeptin neurons), whereas Testagen is proposed to act at the most downstream node (testicular Leydig cells). Kisspeptin-10 has multiple clinical trials demonstrating acute testosterone elevation in men; Testagen has no clinical trial of the synthetic tetrapeptide. Kisspeptin-10 has well-characterized pharmacokinetics and pharmacodynamics; Testagen has neither. The effect size for kisspeptin-10 (50 to 150 percent testosterone increase) substantially exceeds the reported effect for Testoluten (15 to 25 percent), though the comparison involves different study designs and different compounds.

## 12.3 Enclomiphene

**Mechanism:** Enclomiphene is the trans-isomer of clomiphene, a selective estrogen receptor modulator (SERM) that acts as an estrogen receptor antagonist at the hypothalamus and pituitary, blocking estradiol negative feedback and thereby increasing GnRH pulsatility, LH secretion, and downstream testosterone production [38, 39].

**Development trajectory:** Enclomiphene (Androxal) was developed by Repros Therapeutics through Phase 3 clinical trials for secondary hypogonadism (functional hypogonadotropic hypogonadism) in overweight men with low testosterone. The compound received a Complete Response Letter from the FDA in 2015 and was not approved. It is currently approved in some jurisdictions and is widely used off-label [38, 39].

**Novelty:** Low. The SERM mechanism for testosterone elevation has been known since clomiphene citrate was introduced for female infertility in the 1960s. Enclomiphene represents an isomer-purified version.

**Effect size:** Moderate to large. Phase 3 studies demonstrated testosterone normalization (increase from below 300 ng/dL to above 450 ng/dL) in approximately 75 percent of treated men, while preserving spermatogenesis (unlike exogenous testosterone, which suppresses spermatogenesis through HPG axis negative feedback) [38, 39].

**Promising potential:** Moderate. Not FDA-approved despite Phase 3 data. Widely used in clinical practice off-label. Active interest in male reproductive endocrinology.

**Side-effect profile:** Generally well tolerated. Headache, nasopharyngitis, and upper respiratory infection were the most common adverse events in Phase 3 trials, occurring at rates similar to placebo. Theoretical concerns include visual disturbances (a class effect of SERMs) and thromboembolic risk, though neither has been observed at clinically significant rates in enclomiphene trials [38, 39].

**Overall validation:** Moderate to high. Phase 3 clinical trials with large sample sizes and consistent testosterone elevation. Complete Response Letter reflects regulatory concerns (principally regarding the assay used for testosterone measurement) rather than efficacy or safety failure.

**Comparison to Testagen:** Enclomiphene is an oral small molecule with established pharmacokinetics, well-characterized mechanism, and Phase 3 clinical data. Testagen is a research-grade tetrapeptide with no clinical data meeting Western standards. Enclomiphene acts centrally (hypothalamic-pituitary estrogen receptor blockade) whereas Testagen is proposed to act peripherally (testicular gene expression). Enclomiphene produces testosterone elevation of substantially greater magnitude (normalization from hypogonadal levels) than the modest elevations reported for Testoluten. The comparison again highlights the evidence gap.

## 12.4 Epithalon (AEDG)

**Mechanism:** Epithalon is a synthetic tetrapeptide (Ala-Glu-Asp-Gly) from the Khavinson bioregulator family, derived from pineal gland extracts. The principal proposed mechanism is telomerase activation in somatic cells, with additional

chromatin-decondensing and immunomodulatory activities consistent with the general Khavinson bioregulator mechanism [18, 40, 41].

**Development trajectory:** Epithalon is the most extensively studied of the Khavinson synthetic bioregulators. Rodent lifespan studies reported significant lifespan extension (10 to 25 percent) in CBA, SHR, and other mouse strains [18, 27]. Human ex vivo studies demonstrated telomerase activation in peripheral blood cells from aged donors [40, 41]. No controlled human clinical trials meeting Western standards have been conducted.

**Novelty:** Moderate within the Khavinson class. The telomerase activation claim distinguishes Epithalon from the organ-specific bioregulators, though the mechanism remains incompletely validated by Western molecular biology standards.

**Effect size:** Substantial in rodent lifespan models (10 to 25 percent lifespan extension). The ex vivo telomerase activation data in human cells are consistent but do not demonstrate clinical efficacy [18, 40, 41].

**Promising potential:** Low to moderate. No clinical development trajectory. Research-grade material is widely available.

**Side-effect profile:** No adverse events reported in published studies. The general Khavinson bioregulator safety framework applies.

**Overall validation:** Low to moderate. The compound has the most extensive pre-clinical data set of the Khavinson synthetic bioregulators, but lacks clinical validation.

**Comparison to Testagen:** Epithalon differs from Testagen by a single residue at position 1 (Ala versus Lys). The two compounds are structurally the most closely related members of the Khavinson bioregulator panel that target different organ systems (pineal versus testicular). Epithalon has a substantially larger published evidence base, including rodent lifespan studies and human ex vivo telomerase data. Testagen has a smaller evidence base limited to testicular endpoints. The comparison illustrates the within-class variation in evidence maturity among the Khavinson bioregulators.

## 12.5 Human chorionic gonadotropin (hCG)

**Mechanism:** hCG is a 237-amino acid glycoprotein hormone produced by trophoblastic tissue during pregnancy. It shares the alpha subunit with LH, FSH, and TSH and binds the LH/CG receptor on Leydig cells with high affinity, directly stimulating testosterone production through the same cAMP-PKA-StAR signaling cascade activated by endogenous LH [42, 43].

**Development trajectory:** hCG has been used for decades in the treatment of male hypogonadotropic hypogonadism, in the maintenance of intratesticular testosterone during testosterone replacement therapy (to prevent testicular atrophy and preserve spermatogenesis), and in the stimulation of testicular descent in cryptorchidism [42, 43]. It is an established pharmaceutical product with multiple approved indications.

**Novelty:** Low. The compound has been in clinical use for over 50 years.

**Effect size:** Large. Subcutaneous hCG at 1,000 to 5,000 IU two to three times weekly produces substantial testosterone elevation and maintains spermatogenesis in hypogonadotropic men. The effect is direct and dose-dependent [42, 43].

**Promising potential:** Established. Approved pharmaceutical with defined clinical applications and extensive clinical experience.

**Side-effect profile:** Generally well tolerated at standard doses. Adverse events include injection site reactions, headache, mood changes, and gynecomastia (through aromatization of elevated testosterone to estradiol). Rare ovarian hyperstimulation syndrome in women; not applicable in men. Antibody development against hCG has been reported rarely [42, 43].

**Overall validation:** High. Extensive clinical validation across multiple indications. Decades of clinical experience. Well-characterized pharmacokinetics and pharmacodynamics.

**Comparison to Testagen:** hCG is the direct pharmacological comparator for Testagen at the level of Leydig cell stimulation, as both compounds are proposed to act at the testicular level to increase testosterone production. hCG acts through the LH receptor to directly activate the cAMP-StAR steroidogenic cascade; Testagen is proposed to act through epigenetic upregulation of the same cascade components. hCG produces robust, rapid, dose-dependent testosterone elevation; Testagen produces modest, delayed, and incompletely characterized testosterone effects. hCG is

an approved pharmaceutical with comprehensive clinical validation; Testagen is a research-grade peptide with no clinical validation. The comparison is the most directly relevant in this panel and illustrates the distance between the Testagen evidence base and established clinical therapeutics targeting the same endpoint.

## 12.6 Comparator summary

The five comparator compounds span the full range of pharmacological approaches to male testosterone modulation and the full range of evidence maturity. **Gonadorelin** and **hCG** represent the established pharmaceutical reference standards, with decades of clinical use, comprehensive pharmacokinetic and safety data, and approved indications. **Kisspeptin-10** represents the emerging clinical frontier, with a novel mechanism and active clinical investigation but no approved indication. **Enclomiphene** represents the oral small-molecule approach with Phase 3 data. **Epithalon** represents the within-class Khavinson bioregulator comparator.

Testagen occupies the weakest evidence position in this comparator panel. It is the only compound without any clinical trial data meeting Western regulatory standards. It is the only compound without published pharmacokinetic data. It is the only compound whose mechanism has not been independently validated outside the originating research group. Its principal scientific basis is the Khavinson bioregulatory peptide framework, which, while internally consistent and supported by a substantial (though single-institution) body of preclinical data, has not achieved the independent replication and mechanistic confirmation that would support clinical translation by Western standards.

The principal scientific interest of Testagen lies in the novelty of the proposed mechanism (epigenetic modulation of steroidogenic gene expression through direct peptide-DNA interaction), which, if validated, would represent a fundamentally different approach to testosterone modulation compared to the receptor-mediated mechanisms of the other comparators. The validation of this mechanism at the molecular biology level (high-resolution structural characterization of peptide-DNA interactions, CRISPR-based genetic confirmation of target gene involvement, independent replication of the gene expression findings) would be required before Testagen could be considered a credible clinical candidate.

Investigators considering Testagen for research applications should be aware that the compound is at the earliest stage of evidence development among the candidates in this panel, that the evidence originates predominantly from a single re-

search group, and that the clinical comparators (gonadorelin, hCG, enclomiphene, kisspeptin-10) offer substantially better-characterized and more potent approaches to the same testosterone-modulation endpoint.

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