



Review

Physiological and pharmacological overview of the gonadotropin releasing hormone

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ABSTRACT

Gonadotropin-Releasing Hormone (GnRH) is a decapeptide responsible for the control of the reproductive functions. It shows C- and N-terminal aminoacid modifications and two other distinct isoforms have been so far identified. The biological effects of GnRH are mediated by binding to high-affinity G-protein couple receptors (GnRHR), showing characteristic very short C tail. In mammals, including humans, GnRH-producing neurons originate in the embryonic nasal compartment and during early embryogenesis they undergo rapid migration towards the hypothalamus; the increasing knowledge of such mechanisms improved diagnostic and therapeutic approaches to infertility. The pharmacological use of GnRH, or its synthetic peptide and non-peptide agonists or antagonists, provides a valid tool for reproductive disorders and assisted reproduction technology (ART).

The presence of GnRHR in several organs and tissues indicates additional functions of the peptide. The identification of a GnRH/GnRHR system in the human endometrium, ovary, and prostate has extended the functions of the peptide to the physiology and tumor transformation of such tissues. Likely, the activity of a GnRH/GnRHR system at the level of the hippocampus, as well as its decreased expression in mice brain aging, raised interest in its possible involvement in neurogenesis and neuronal functions. In conclusion, GnRH/GnRHR appears to be a fascinating biological system that exerts several possibly integrated pleiotropic actions in the complex control of reproductive functions, tumor growth, neurogenesis, and neuroprotection.

This review aims to provide an overview of the physiology of GnRH and the pharmacological applications of its synthetic analogs in the management of reproductive and non-reproductive diseases.

1. Introduction

Gonadotropin-Releasing Hormone (GnRH, formerly known as Luteinizing-Hormone-Releasing Hormone, LHRH) was isolated and sequenced in 1971 by Andrew Schally [1], then awarded the Nobel Prize for Medicine in 1977 together with Roger Guillemin and Rosalyn Yalow.

GnRH is a hypothalamic factor belonging to the hypothalamic-releasing hormones family, and it is functionally similar to corticotrophin-releasing hormone, growth hormone-releasing hormone, and thyrotrophin-releasing hormone. Chemically, GnRH is a decapeptide; it is released from a limited number of hypothalamic neurons scattered in the mediobasal hypothalamus and exerts the role of the main central regulator of reproductive functions as part of the so-called Hypothalamo-Pituitary-Gonadal (HPG) axis [2,3] (Fig. 1).

Intricate time and space scales characterize the function of the HPG axis. The physiological processes characterizing the HPG axis occur in

millisecond (neuronal) timescale as well as circannual rhythmicity and spacing from molecules to organs. From this point of view, the signals from the internal and external environment, elaborated by high brain centers, are integrated at the hypothalamic level in the neuronal circuits forming the 'GnRH system'. GnRH-producing neurons project and ramify into numerous terminals that appose to hypothalamic blood capillaries creating the hypophyseal portal system and releasing GnRH directly into the bloodstream addressed to the anterior pituitary [4].

A low and high-frequency GnRH pulsatile secretion forwards the instructions to the pituitary gland to generate alternative responses. At the pituitary level, these signals are once more integrated through the activation of specific GnRH receptors (GnRHR) [5] with consequent differential production and release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), which, in turn, induce the response of the gonads (effectors), ovary or testis, with the activation of specific processes, like folliculogenesis, spermatogenesis and

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steroidogenesis.

The gonadal responses generate further information flows by sex steroid (estrogens and progesterone, or androgens) and polypeptidic (e. g., inhibits, activins, follistatin) hormones that feedback in negative and positive modalities at the different integrative centers. Gonadal steroids also exert their imprinting actions on sex phenotype, sexual/reproductive behavior, and other non-reproductive functions [6].

Differential regulatory effects of the ovarian steroids on GnRH secretion and pulsatility have been assessed in many mammals and women: progesterone exerts an inhibitory action while estradiol can have both stimulatory and inhibitory effects, depending upon the stage of the menstrual cycle [7]. In contrast, androgens exert only a negative regulation on gonadotropin secretion.

An impaired development and function of GnRH neurons is the leading cause of central hypogonadotropic hypogonadism (CHH, also classified as idiopathic central hypogonadism, ICH) [8]; CHH is a rare disease (1:8000 males and 1:40 000 females) with a complex pathogenesis; it may be either congenital or acquired and secondary to hypothalamic or pituitary lesions. It is caused by insufficient GnRH stimulation (GnRH deficiency) of an otherwise normal pituitary–gonadal axis, which leads to infertility. The disease is characterized by low levels of sex steroids (testosterone or estradiol) in the presence of low/normal levels of gonadotrophins (LH and FSH) and can be associated with a normal or a defective sense of smell, which identify, respectively, normosmic CHH or Kallmann syndrome (KS).

Although GnRH isoforms have been identified, this review will mainly deal with the first recognized GnRH form due to its well-established use in the clinical pharmacology of its analogs.

1.1. GnRH, gene, and distribution

In humans, the GnRH gene is located as a single copy on the short arm of chromosome 8 (8p11.2-p21); it contains three introns and four exons [9] for a total of 5,783 nucleotides (Fig. 2).

The gene encodes for a 92- amino acids (AA) proGnRH that undergoes post-translational processing by enzymatic cleavage and further modifications at the level of the secretory granules. The prohormone is organized into a) a 23-AA signal peptide involved in intracellular trafficking and secretion, b) the GnRH peptide, c) a proteolytic processing site, and d) a secreted 56-AA peptide (GnRH-associated peptide, GAP), with still unclear functions (Fig. 2) [2].

Chemically, GnRH is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) presenting a cyclized glutamine (pyroGlu) at the N-terminal and a glycine-amide residue at C-terminal that confer some resistance to terminal peptidases (Table 1) [10]. GnRH can be cleaved in three major sites, namely PyroGlu¹-His², Tyr⁵-Gly⁶, and Pro⁹-Gly¹⁰, by pyroglutamate aminopeptidase, endopeptidase, and post-proline-cleaving enzyme (Fig. 2) [11]. The N-terminal (pGlu-His-Trp-Ser) and C-terminal (Pro-Gly-NH₂) AA sequences are involved in receptor binding and activation (Fig. 2) [10].

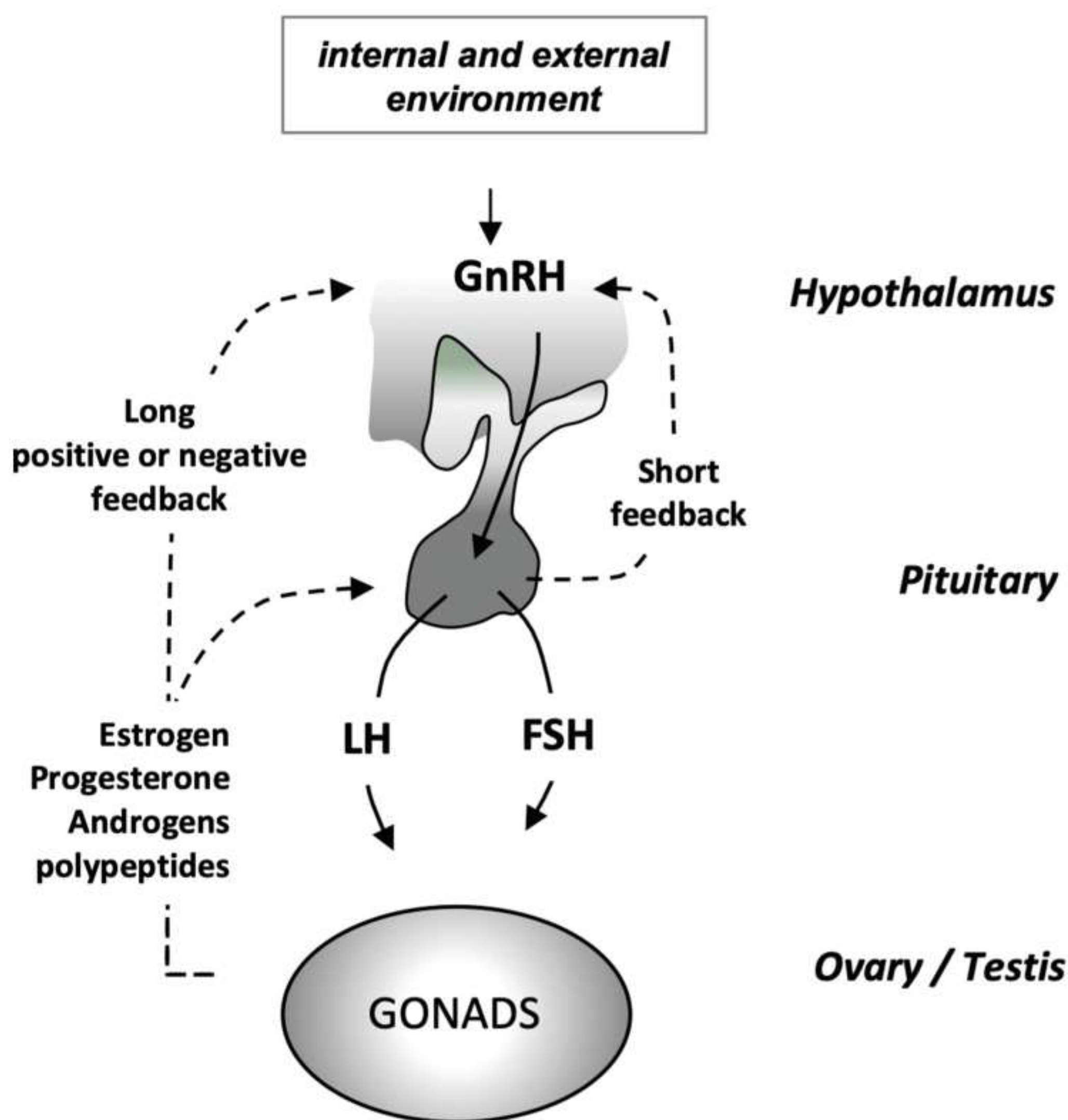


Fig. 1. Schematic representation of the hypothalamo-pituitary–gonadal (HPG) axis. The decapeptide GnRH produced by hypothalamic neurons exerts direct control on gonadotropin (LH and FSH) synthesis and secretion. These, in turn, stimulate the gonads to carry out their reproductive and endocrine functions. Short and long feedback effects regulate the secretion of GnRH itself.

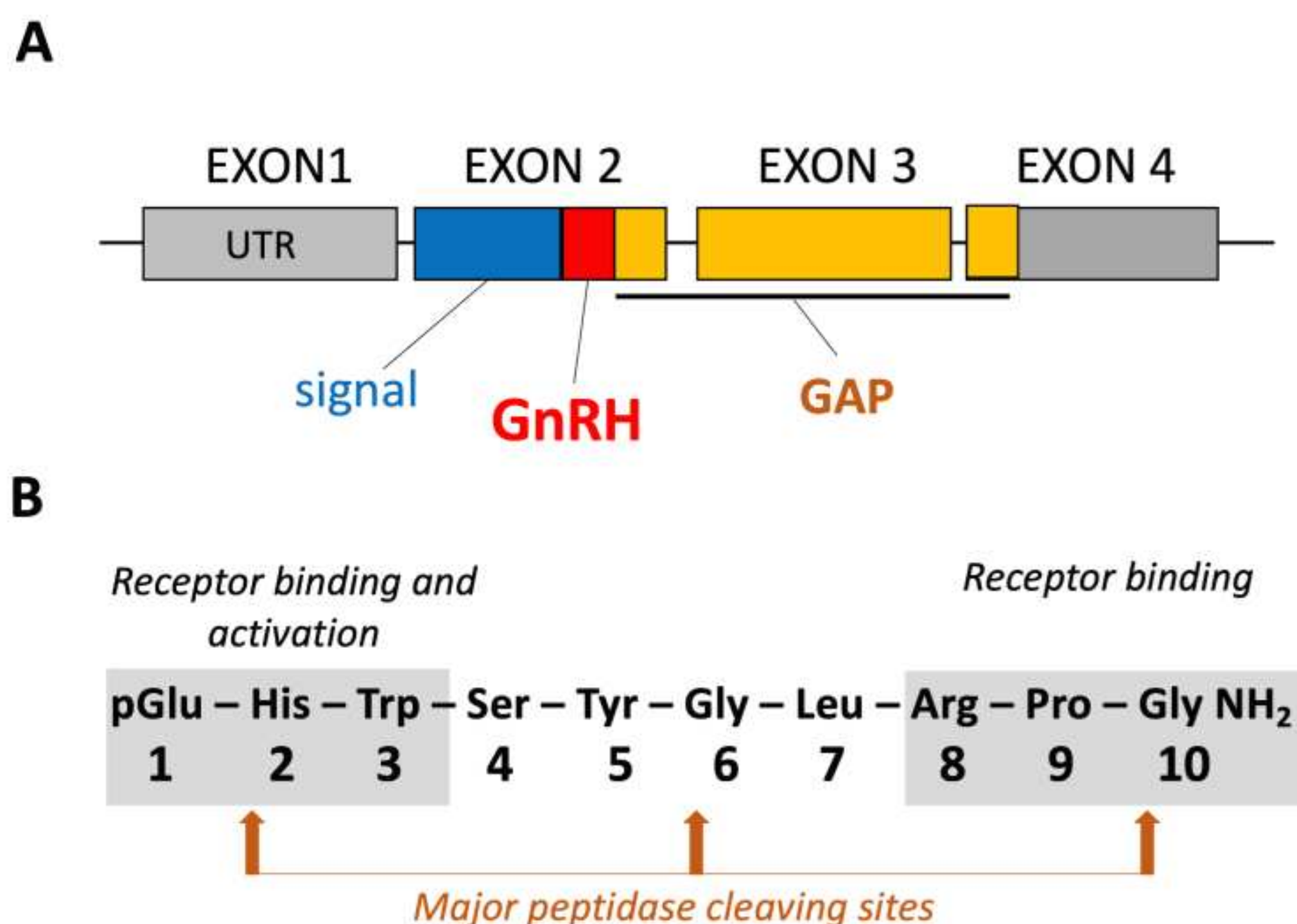


Fig. 2. Schematic representation of the GnRH gene (A) and GnRH peptide (B). A) The gene encodes for a 92- amino acid (AA) proGnRH mainly organized into a 23-AA signal peptide (signal), the GnRH decapeptide (GnRH) and a secreted 56-AA peptide (GnRH-associated peptide, GAP). B) The amino acid sequences of mature GnRH; the AA residues involved in receptor binding and activation as well as the major bonds cleaved by peptidase are indicated.

Table 1

Amino acid sequences of natural GnRH isoforms.

GnRH (also GnRH-I)	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -Gly ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
GnRH-II	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -His ⁵ -Gly ⁶ -Trp ⁷ -Tyr ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
GnRH-III (lamprey)	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -His ⁵ -Asp ⁶ -Trp ⁷ -Lys ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂

In red the modified amino acid residues with respect to GnRH-I.

1.2. GnRH isoforms

In addition to the first characterized GnRH form (named GnRH-I), two other distinct natural GnRH isoforms have been identified and named GnRH-II and GnRH-III (Table 1) [12]. Because of this, the first identified GnRH may also be indicated as GnRH-I. The three GnRH isoforms are supposed to be evolved from two rounds of genome-wide duplication [13]. All share the AA sequences for receptor binding and activation (Table 1 and Fig. 2).

The GnRH-II isoform has been identified in vertebrates and is commonly referred to “chicken GnRH-II” or GnRH-II; it is highly conserved from fish to mammals, including humans [10] where it is encoded by a gene located on chromosome 20 [14]. It shows a 70% of homology with GnRH-I and differs from it for the His⁵, Trp⁷, and Tyr⁸ residues [15]. Studies in primates (*Macaca mulatta*) show that GnRH-II is expressed in cell populations different from those expressing GnRH-I [16]; in particular, it is produced in specific nuclei of the central nervous system (CNS) like supraoptic, paraventricular and suprachiasmatic nuclei. GnRH-II shows different effects from those exerted by GnRH-I, acting as a neuromodulator of the sexual behavior [10]; however, it may also exert a permissive regulation of female reproductive behavior based on body energy status and an effect on food intake [17].

Although this isoform may also be expressed in the mediobasal hypothalamus, and reach pituitary gonadotrope cells [10], it is unlikely it could play a role as the primary regulator of gonadotropin release in primates. In fact, even though it has been observed that GnRH-II may

stimulate gonadotropin secretion through type I GnRHR, it has also been reported that GnRH-II and GnRH-I producing neurons show different responsiveness to estradiol leading to stimulation of GnRH-II and inhibition of GnRH-I gene expression. The possible different but coordinated action of the two distinct GnRH neuronal subpopulations in the control of fertility has been hypothesized [18,19].

GnRH-II was also detected in peripheral organs, like the endometrium, ovary, and placenta [20,21]. In such structures, GnRH-I and II show similar functions in regulating cell proliferation and mediating ovary and placenta hormonal secretion as autocrine/paracrine factors.

However, since GnRH-II is more potent than GnRH-I in peripheral tissue, but less potent in gonadotropin release, the ‘ligand-induced selective signaling’ (LiSS) theory has been proposed. This hypothesize that the two peptides may stabilize different conformations of the type I GnRHR receptor leading to its differential coupling with intracellular pathways [22].

In 1993, a third form of GnRH (GnRH-III) has been isolated from the sea lamprey brain (*Petromyzon marinus*); it shows a 60% of homology with GnRH-I and an additional substitution (Asp⁶) respect to GnRH-II (Table 1) [23]. In the lamprey, GnRH-III plays a crucial role in controlling gametogenesis and steroidogenesis [24]. In human GnRH-III is not expressed, but it may specifically bind to GnRH-I receptors on human cancer cells, exerting a significant anticancer effect on different tumors, but it shows negligible activity on gonadotropin secretion [25].

1.3. GnRH receptors (GnRHRs)

The GnRHR was cloned from pituitary cells from several species. The human pituitary GnRHR (also named type I GnRHR) present on gonadotrope cells is encoded by a gene located on chromosome 4 (4q13), composed of three exons and two introns [26] and translated in a 328-AA protein. It belongs to the family of rhodopsin-like G protein-coupled receptors (GPCR) with the classical seven transmembrane domains and an extracellular amino-terminal domain (35 AA) with two putative glycosylation sites [27]. The receptor is characterized by the presence of a very short carboxy-terminal cytoplasmic tail (C-tail, 1–2 AA), a region involved in desensitization and internalization of G protein-coupled receptors; consequently, the short C-tail confers to GnRHRs an absent desensitization, a slow internalization, and a lack of agonist-induced phosphorylation or binding to beta-arrestin [28–33].

Pituitary GnRHRs are canonically coupled to the $\alpha_{q/11}$ ($G_{\alpha q/11}$) subunit of G protein, but they may also activate α_s ($G_{s/cAMP}$) subunit, suggesting a possible interaction between the two signaling pathways in the complex control of the production of pituitary gonadotropins [34]. The initial response of gonadotropes to GnRH, or its analogs, seems to involve the $G_{s/cAMP}$ pathway, providing the transmission of GnRHR signals to the nucleus with the consequent cell activation [35].

However, the GnRH-induced dissociation of $G_{\alpha q/11}$ activates the phospholipase C β (PLC β), leading to intracellular accumulation of diacylglycerol (DAG), with stimulation of the protein kinase C (PKC), and inositol 1,4,5-triphosphate (IP $_3$), that triggers the release of Ca $^{2+}$ from intracellular stores, to activate gonadotropin synthesis and secretion. Moreover, the $G_{\alpha q/11}$ signal undergoes rapid desensitization. Recent results from selective gene knockdown in mice confirm that $G_{\alpha q/11}$ is mainly involved in gonadotropin production, while $G_{s/cAMP}$ signaling could be involved in the enhancement of GnRH secretion occurring during the ovarian cycle or after gonadectomy [34].

GnRHR may also activate downstream signaling pathways involving the mitogen-activated protein kinase (MAPK) cascades, including ERK, p38 MAPK, and JNK; the subsequent activation of phospholipases D and A2 leads to an additional and prolonged action of PKC [36].

The efficient activation of pituitary GnRHRs requires a pulsatile stimulation by GnRH; on the contrary, continuous exposure to GnRH leads to desensitization of GnRH-stimulated gonadotropin secretion; this is associated with receptor down-regulation and internalization [37–39] that does not account for the magnitude of reduced response of gonadotrope cells [40]. In fact, these receptors do show agonist-induced internalization, but this is much slower [28–33].

Therefore, the desensitization of gonadotrope cells to the GnRH signal could reflect downstream adaptive responses, even in the absence of receptor desensitization. For instance, *in vitro* studies have shown that sustained stimulation of gonadotrope cells with GnRH causes the relatively rapid desensitization of Ca $^{2+}$ responses that can reflect a reduction in the efficiency of IP $_3$ to mobilize Ca $^{2+}$ from these stores [41,42]. On the other hand, long-term treatment of gonadotrope cells with GnRH-agonist (GnRH-a) and GnRH-antagonist (GnRH-ant) similarly caused a marked inhibition of GnRHR mRNA expression [43], gonadotrophin synthesis and depletion of releasable gonadotrophin pools [44]. Thus, it has been proposed that the type I GnRHR differs from other G protein-coupled receptors for a novel form of post-receptor desensitization [42]. From a pathological point of view, mutations of GnRHR were found to be associated with CHH [45].

The demonstration of a specific receptor for GnRH-II (a ‘type II’ GnRHR) was first provided in non-human primates [46]. It belongs to the GPCR receptors family but, unlike type I GnRHR, has an intracellular tail carrying several potential phosphorylation sites involved in the rapid internalization and desensitization processes [46,47]. The gene for the putative human type II GnRHR shows a frameshift in coding exon 1 and a premature internal stop codon in the sequence corresponding to the extracellular loop, suggesting the absence of a functional full-length type II GnRHR in humans [10,48]. In fact, the effects exerted by GnRH-

II, or its agonists, on the pituitary or peripheral tissues (e.g., anti-proliferative/antimotility effects on uterine endometrium and prostate), are mediated by the activation of the type-I GnRHR [49]. Likewise, also the effects of GnRH-III on human cells occur through the activation of type I GnRHR [50,51].

1.4. GnRH neuron development and distribution

Most vertebrates have not only multiple GnRH genes but also different GnRH neurons. The best-studied GnRH neuron development is referred to as mammalian GnRH-I neurons [12]. These neurons are not organized in discrete brain nuclei but are instead distributed throughout the mediobasal hypothalamic region.

GnRH neurons emerge in the embryonic frontonasal mesenchyme (nasal placode), a region that also gives rise to olfactory sensory neurons, and take on a peculiar long-distance migration along a path composed of putative vomeronasal/terminal nerve fibers to enter the brain and reach their final destination in the preoptic area of the hypothalamus [12].

In mice, GnRH-I neurons start invading the brain around embryonic day (E) 12.5 and conclude their migration to the basal forebrain around E 16.5 [52]; in adults, about 1,500 GnRH expressing neurons populate the hypothalamic region.

In humans, GnRH-I neurons start their migratory journey around the gestational week (GW) 5 and 6, and at GW 11–12 the majority of them are approaching the hypothalamic region. The overall number of GnRH immunoreactive neurons in humans is significantly higher than in rodents; about 10,000 GnRH neurons are present in the whole human brain during fetal life, with about 2,000 neurons located in the hypothalamus and 8,000 neurons distributed in widespread brain areas that may account for the non-reproductive functions of GnRH [53].

At the destination, the GnRH-I neurons project-specific contacts with the capillaries of the vascular pituitary portal system undertaking synaptic connections with several neuronal networks; its activity is fine-tuned by crosstalk of neurotransmitters, glial-derived growth factors, neuropeptides, genetic and/or epigenetic factors, and by the hormonal milieu [54,55].

An altered migration of GnRH neurons was first observed to be associated with mutations of *ANOS1* gene in the X-linked form of Kallmann’s syndrome (KS) [56,57]. Studies on the etiopathogenesis of KS, and other CHH, led to the identification of several genes/factors involved in the development of GnRH neurons. In fact, mutations found to be linked with defects in the emergence of GnRH neurons from the nasal placode were found in fibroblast growth factor 8 (FGF8) and FGF receptor 1 (FGFR1) genes, the so-called “Fgf8 synexpression genes”, as well as in Paired Box gene 6 (Pax6), and the in chromodomain helicase DNA binding protein 7 (CHD7) gene. In particular, FGF8 and its receptor FGFR1 are important for specifying GnRH neurons [12]. The second set of mutations identified in CHH includes genes involved in cell adhesion, neuronal pathfinding, and cell migration, such as anosmin-1, nasal embryonic LHRH factor (NELF), Semaphorin 7A (SEMA7A), CHD7, SEMA3A, and SRY-Box Transcription Factor 10 (SOX10) [12,58]. The third set of mutations involves genes related to the migration of GnRH neurons and includes Neuron-derived neurotrophic factor (NDNF), stromal cell-derived factor 1 (SDF1)/chemokine receptor (CXCR-4), GABA, Hepatocyte growth factor (HGF)/Met receptor, PlexinB1, and Cholecystokinin (CCK) [12]. However, an overall assessment of the complex interactions between these factors is still to be clarified. The application of new techniques of gene sequencing (Whole Exome Sequencing and Next Generation Sequencing), applied to genotype/phenotype correlation, will contribute elucidating the roles of the regulators of GnRH neuron developmental program in cell fate specification, migration, survival, and function [59,60].

2. GnRH secretion and actions

2.1. GnRH secretion

The release of GnRH into the hypothalamic-hypophyseal portal vessels must occur in a pulsatile manner to induce a synchronized gonadotropins secretion [61–63].

In primates, including humans, the GnRH neuronal network responsible for peptide release is fully active during late fetal maturation and in the early neonatal period; it becomes quiescent during childhood until it will be fully reactivated at puberty to induce the onset and the maintenance of reproductive functions through life [2].

The release of GnRH is under the control of a complex neuronal network, involving hypothalamic and extra-hypothalamic inputs, and of peripheral generated signals [64]. The central and peripheral signals involved in the modulation of GnRH secretion have been extensively investigated and different stimulatory (e.g., kisspeptin, norepinephrine, and neuropeptide Y, estrogens), or inhibitory (e.g., endogenous opioids, interleukin-1, estrogens, progesterone, androgens) factors have been identified in humans. Finally, GnRH itself may regulate its own secretion through an ultra-short feedback loop on hypothalamic neurons [65].

Although in animal models GnRH can be assayed in the blood of pituitary portal vessels, in humans it can be only measured in peripheral blood, but it does not accurately reflect hypothalamic peptide secretion due to its short half-life; however, since the serum fluctuation of glycoprotein free α -subunit (FAS) of LH reflects GnRH pulse generator activity [66], what follows as GnRH pulses in humans refers to plasma LH or FAS measurement.

In the human adult male, GnRH pulses occur approximately every 120 min; more complicated is the pattern of pulsatile GnRH release in a woman since it shows variations both during the different reproductive stages and the ovulatory cycle; in healthy women the pulse frequency range from approximately 95 min, in the early follicular phase, to 60–70 min in the periovulatory period [67].

2.2. Action of GnRH on pituitary gonadotropes

Although GnRH acts as a *releasing hormone* for the two gonadotropins, they are differentially regulated according to the capacity of pituitary gonadotrope cells to decode the GnRH signals. As cited above, a pulsatile release of GnRH is mandatory for efficient activation of gonadotrope cells, in fact, a continuous infusion of GnRH induces a rapid, but reversible, shutdown of the reproductive axis by suppression of both LH and FSH release [68]. This dual effect may involve GnRHRs modulation; a short-term pulsatile treatment with GnRH results in pituitary GnRHRs up-regulation, whereas a prolonged high-dose treatment induces a rapid uncoupling of the GnRHR from its intracellular signaling molecules and a slow-down-regulation of the receptors [69,70].

The rhythmic stimulation of pituitary GnRHR may activate the different G protein subunits with which it is coupled, providing the basis for the decodification of GnRH signals of different frequencies and amplitudes.

Under a pulsatile GnRH regimen, the $G_{\alpha s}$ cAMP mediated-signaling is early activated together with that of $G_{\alpha q/11}$, but the latter undergoes rapid desensitization, suggesting that $G_{\alpha s}$ and the $G_{\alpha q/11}$ pathways might mediate an adaptive and a desensitization response, respectively, to pulses of GnRH [71]. *In vitro* studies have shown that several intracellular signal cascades (MAPKs; see above), as well as transcription factors, are involved in driving the gonadotrope-specific expression of LH and FSH genes [63]. LH expression is induced by high-frequency GnRH pulses with the consequent activation of steroidogenic factor 1 (SF1) and early growth response 1 (EGR1) [72]. On the contrary, FSH expression is induced by low-frequency GnRH pulses which activate EGR1 to a lesser extent, leading to a prevalent action of co-repressors for LH promoter and activating AP-1 transcription factor family members (c-fos, c-jun) [73].

Because of such mechanisms, it is evident that efficient therapeutic application of GnRH to restore gonadal functions requires an intermittent GnRH delivery [74,75]; conversely, a continuous administration of GnRH leads to the so-called ‘medical castration’. Of pharmacological interest are the mechanisms of down-regulation of the GnRH signaling induced by continuous tonic exposure to the peptide or its analogs [76]. In fact, with continuous GnRH exposure, the early activated $G_{\alpha s}/cAMP$ pathway undergoes to a quick deactivation; contrary to what has been described in response to pulsatile administration, $G_{\alpha q/11}$ pathway does not desensitize but remains constantly activated, altering the fine-tuning control of gonadotropin synthesis and release [35].

2.3. Effects of GnRH on extra pituitary tissues

First demonstrated in the human placenta, GnRHR were found to be expressed, at the mRNA and protein level, in normal and pathological female (ovary and endometrium) and male (prostate) reproductive system [3,77,78]. Prostatic GnRHRs appear to be different from the pituitary ones showing a lower binding affinity for GnRH and its analogs [79,80]; however, GnRHR with high affinity for GnRH have been described in the endometrium and ovary [81,82].

Likely, GnRH-I was found to be expressed and immunodetected in these tissues and GnRH-II peptide is also present in placenta, ovary and prostate [83]. Finally, functional studies confirm the presence of a GnRH/GnRHR system suggesting a possible autocrine/paracrine action of the decapeptide in many structures of the reproductive system [3,77,78]. Of interest, GnRH analogs acting as antagonists on the pituitary receptors, show an agonist-like biological effect in tumors of the reproductive tissues [84,85]. Pituitary and peripheral GnRHR also differ in terms of intracellular signaling pathways. GnRHR in tumoral reproductive tissue has been found to be coupled to the $G_{\alpha i}$ pathway, whose activation leads to a reduction of intracellular cAMP levels with the consequent activation of a signaling cascade, including MAPK, phosphatidylinositol-3-kinase (PI3K), and phosphotyrosine phosphatase [22]. This pathway is involved in antitumoral effects of GnRH analogs found in several cancer cell lines [86,87]. However, the different action of GnRH and its analogs in peripheral tissues is still an open question and the LiSS theory may still be evoked [22].

It is possible that in extra pituitary cells GnRHR might assume different conformations due to alternative post-translational processing and that GnRH analogs might bind such receptors in a different way, triggering alternative intracellular signaling pathways and, consequently, biological effects [3].

3. Pharmacology of GnRH and its analogs

From a historical point of view, a major advance in GnRH pharmacology emerged from the work of Knobil and coworkers who first highlighted that continuous, rather than pulsatile, administration of exogenous GnRH induced a loss of response of pituitary gonadotrope cells (Fig. 3) [68] suggesting its use to both stimulate or inhibit the HPG axis.

In addition to the pulse frequency, appropriate dosages and routes of administration of GnRH are pivotal for the successful outcome of the treatment. For instance, sub-cutaneous (s.c.) administration can be considered even though it requires higher doses than the intravenous (i.v.) route.

The GnRH pulse frequency measured during the menstrual cycle was taken as a reference protocol to stimulate the HPG axis [88]. Various studies have shown that after 1 μ g i.v. pulse, GnRH will reach a peak of serum concentration between 200 and 260 pg/mL within 4 min [89], in the range of the physiological concentration found in pituitary portal blood (40–2000 pg/mL) [90].

The administration of 1 μ g GnRH i.v. every 90 min in the early follicular phase, and every 60 min in the periovulatory period, was found to induce a significant rise of plasma GnRH/gonadotropins and a

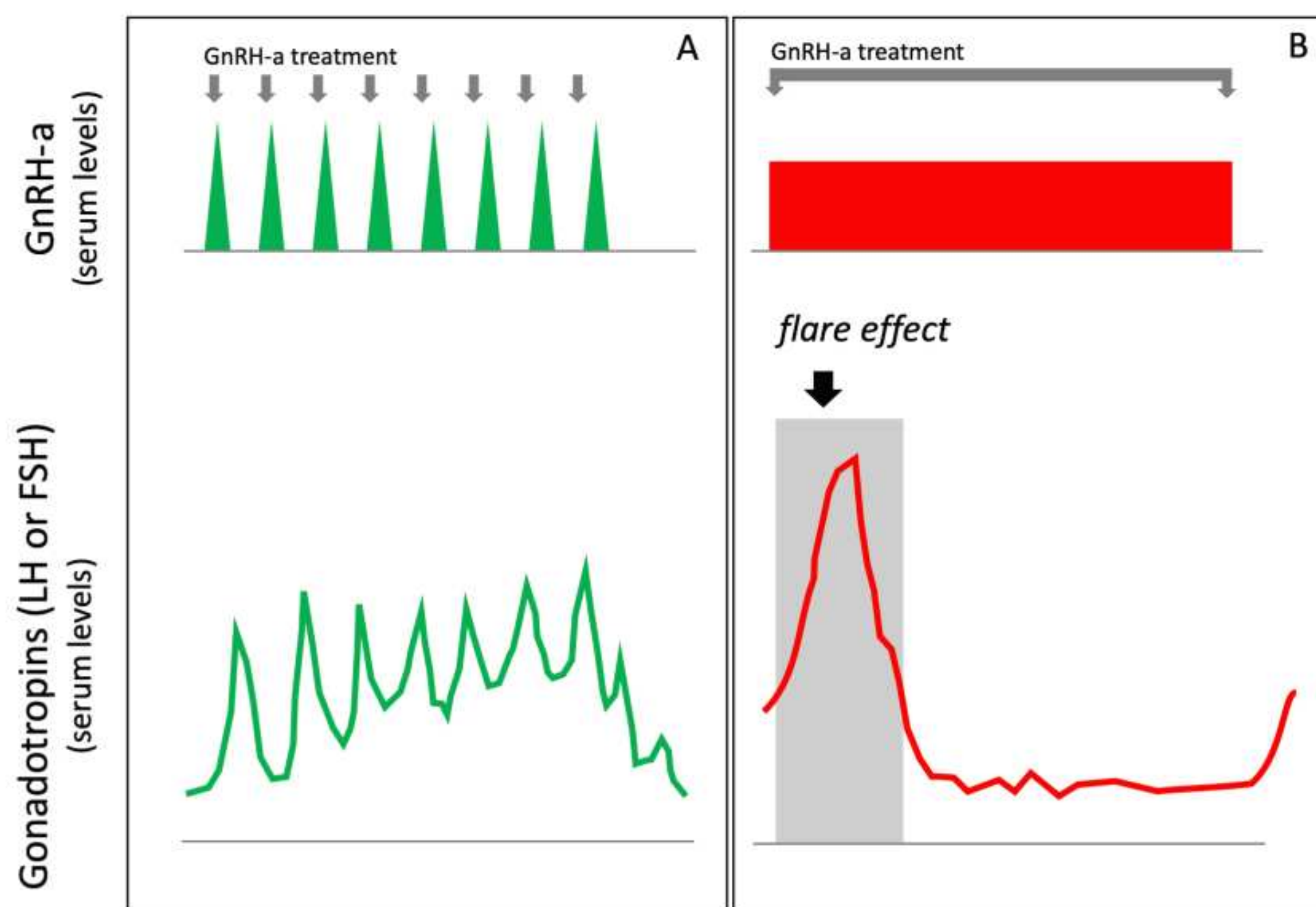


Fig. 3. Schematic representation of the response, in term of gonadotropin serum levels, to different regimens of GnRH and GnRH-a administration. A) Pulsatile i.v. administration is able to induce efficient episodic releases of FSH and LH; B) continuous (i.m. or s.c., daily or depot) administration induces an intense reduction of serum gonadotropin levels and a reproductive axis shutdown. The administration of GnRH superagonists may produce an initial intense stimulation of the pituitary GnRH receptor and the characteristic transient serum gonadotropin burst (*flare effect*).

final high outcome rate in terms of ovulation (more than 90%), pregnancy as well as puberty induction [91–94]. If the interpulse period is increased to 120 min, the ovulation rate drops to 70% [91].

In the early nineties, the hormonal effects of i.v. pulse dosages at optimal time intervals were more clearly defined [67]. In a 50 kg patient, a low dosage of GnRH (25 ng/kg, 1.25 µg/pulse) induced a peak of estradiol, the corpus luteum function, and an ovulatory rate lower than normal. At 75 ng/kg (3.75 µg/pulse for the same 50 kg patient), the above parameters were almost normal; meanwhile, at 100 ng/kg (5 µg/pulse for a 50 kg patient), the ovulatory rates were maintained (93%), but estradiol and progesterone levels were higher than in the ovulatory cycle.

It appears so clear that, pulsatile therapy with natural GnRH must be accurately evaluated regarding the physiopathology of infertility.

Native GnRH is efficiently absorbed after i.v., s.c., intramuscular (i.m.), nasal, or sublingual administration [95], but while the i.v. route is able to induce efficient episodic releases of FSH and LH, s.c. administration results in a slower rise of the two gonadotropins but, above all, without a normal pulsatile pattern.

GnRH is promptly degraded in the peripheral circulation, with a half-life of 2–8 min [96], mostly due to the degradation of the glycine-leucine bond between AA⁶ and AA⁷.

All these data also indicate that a pharmacological intervention using native GnRH needs an accurate pharmacokinetic approach.

3.1. GnRH agonists

The short half-life of native GnRH led to the development of peptide agonists (GnRH-a) (Table 2). In order to preserve the capacity of these analogs to interact with the receptor, the sequence of the N-terminal and the C-terminal domains were kept as similar as possible to GnRH decapeptide [97]. Synthetic GnRH-a have been designed with a modification of Gly⁶, which is replaced with a D-AA, that increases plasma half-life compared to GnRH. An increased affinity for GnRHs was

Table 2

Amino acid sequences of GnRH agonists and antagonists.

GnRH	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -Gly ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
Agonists	
Buserelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Ser(tBu) ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -NH ₂
Goserelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Ser(tBu) ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -AzaGly ¹⁰ -NH ₂
Leuprolide	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Leu ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -NH ₂
Triptorelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Trp ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
Deslorelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Trp ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -NH ₂
Nafarelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-Nal ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
Histrelin	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -Tyr ⁵ -D-His(Bzl) ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -NH ₂
GnRH-II-a	pGlu ¹ -His ² -Trp ³ -Ser ⁴ -His ⁵ -D-Lys ⁶ -Trp ⁷ -Tyr ⁸ -Pro ⁹ -Gly ¹⁰ -NH ₂
Antagonists	
Abarelix	Ac-D-Ala ¹ -D-Cpa ² -D-Ala ³ -Ser ⁴ -Tyr ⁵ -D-Asp ⁶ -Leu ⁷ -Lys(iPr) ⁸ -Pro ⁹ -D-Ala ¹⁰ -NH ₂
Cetrorelix	Ac-D-Nal ¹ -D-Cpa ² -D-Pal ³ -Ser ⁴ -Tyr ⁵ -D-Cit ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -D-Ala ¹⁰ -NH ₂
Ramorelix	Ac-D-Nal ¹ -D-Cpa ² -D-Trp ³ -Ser ⁴ -Tyr ⁵ -D-Ser(Rha) ⁶ -Leu ⁷ -Arg ⁸ -Pro ⁹ -AzaGly ¹⁰ -NH ₂
Degarelix	Ac-D-Nal ¹ -D-Cpa ² -D-Pal ³ -Ser ⁴ -Aph(Hor) ⁵ -D-Aph(Cba) ⁶ -Leu ⁷ -Lys(iPr) ⁸ -Pro ⁹ -D-Ala ¹⁰ -NH ₂
Ganirelix	Ac-D-Nal ¹ -D-Cpa ² -D-Pal ³ -Ser ⁴ -Tyr ⁵ -D-hArg(Et) ⁶ -Leu ⁷ -hArg(Et) ⁸ -Pro ⁹ -D-Ala ¹⁰ -NH ₂
Triptorelin (GnRH-II-ant)	Ac-D-Nal ¹ -D-Cpa ² -D-Pal ³ -Ser ⁴ -Tyr ⁵ -D-Cit ⁶ -Trp ⁷ -Tyr ⁸ -Pro ⁹ -D-Ala ¹⁰ -NH ₂

Abbreviations: tBu: *tert*-butyl; Et: ethylamide; AzaGly: aza-glycine; Bzl: 1 benzyl; Ac: acetyl; Cpa: chlorophenylalanine; Pal: 3-pyridylalanine; Cit: citrulline; Aph: 4-aminophenylalanine; Hor: L-hydroxyrotyl; Cba: carbamoyl; iPr: isopropyl; Nal: 2-naphthylamine; hArg: homoarginine; D-Ser(Rha): O-(6-deoxy-α-L-mannopyranosyl)-D-serine. In bold the modified residues with respect to mammalian GnRH.

instead obtained with the deletion of Gly¹⁰-amide and the addition of an ethylamide residue to Pro⁹, [98]. Buserelin, goserelin, leuprolide, and triptorelin are the most representative GnRH-a; all of them present a substitution of Gly⁶ respectively with D-Ser (tBu), D-Ser (tBu), D-Leu, and D-Trp residues (Table 2).

The first three compounds are nonapeptides and have, respectively, Aza-GlyNH₂ and N-ethylamide substitutions at C-terminal, whereas triptorelin is a decapeptide with the original Gly¹⁰NH₂ residue. Other agonists become then available, such as deslorelin (D-Trp⁶ and a final N-ethylamide), nafarelin (D-2-naphthylamine⁶), and histrelin (D-His-1-benzyl⁶ and final N-ethylamide) [99].

The agonist analogs of GnRH are characterized by high potency (up to 200 times that of the native GnRH and so-termed 'superactive'), prolonged action, and low toxicity.

Generally, GnRH-a are mainly indicated for continuous administration when the repression of the HPG axis is requested (i.e., precocious puberty, endometriosis, procedures of *in vitro* fertilization, prostatic carcinoma).

It is worth mentioning that GnRH-a has been recommended as an initial treatment for adolescents diagnosed with gender dysphoria [100]. By irreversibly blocking pubertal development, GnRH-a may avoid distress caused by unwanted pubertal changes and give the child and their family more time in which to explore the possibility of dealing with gender-affirming hormone therapy. Nevertheless, this prescription is currently debated and under a deeper investigation [101,102].

However, due to their stability and high potencies, the administration of GnRH-a induces an initial transient intense stimulation of the pituitary GnRHR with an increase of gonadotropin secretion that characterizes the so-called *flare-up* response (also referred to as *flare effect*) (Fig. 3); this response is followed by GnRHR desensitization and reproductive axis shutdown with consequent reversible medical castration [103].

3.1.1. Pharmacokinetic and pharmacodynamic of GnRH and its agonists

For reference, the pharmacokinetic parameters of buserelin, triptorelin, goserelin, and leuprolide are reported in Table 3. For a better comparison, the data of s.c. treatments, as the most used, are reported [104–111].

A very important aspect of the bioactivity of superagonists is their resistance to enzymatic degradation due to the absence of suitable cleavage sites present in these molecules.

After i.v. administration of native GnRH, or GnRH-a, a biphasic plasma concentration kinetic with a rapid (related to the distribution phase) and a slow (related to the elimination phase) component, consistent with a two-compartment model, was observed [112].

The first compartment is vascular and rapidly exchangeable

extravascular fluid, whereas the second compartment, with a slower clearance, cannot be identified with a particular organ, but it may incorporate a group of tissues that exchange at the same speed with plasma as demonstrated through radiolabel compounds; the main site of incorporation of radiolabeled buserelin are muscle, skin, blood, liver, and bowel [113].

The serum binding capacity of the GnRH is low and comparable to its agonists except for nafarelin that is about 4 times higher (80%) [99]. As expected, agonists show a higher half-life and a lower metabolic clearance than native GnRH [112].

An important aspect that may affect the pharmacokinetics of GnRH-a is the route of administration. For instance, after s.c. administration, the t_{1/2} distribution phase of triptorelin is tenfold higher, and the metabolic clearance rate is threefold lower with respect to i.v. infusion, as a consequence of the progressive release of the analog from the injection site [99]. Concerning bioavailability, s.c. route ranges between 66 and 90% (vs. i.v. 100%), whereas inhalator administration can achieve a bioavailability of 28% and the sublingual route of only 2% [99].

Some depot formulations containing embonate or pamoate salts have been designed to increase the time of release [115]. Conjugation with polyethylene glycol (PEGylation) is an alternative method to increase the circulating half-life of a peptide/protein-based drug. PEGylation reduces peptide renal clearance, and proteolytic degradation, increasing its stability. For instance, PEGylation of leuprolide increases its half-life from 0.43 h to 0.53 h for PEG2k-Leu, or to 1.51 h for PEG5k-Leu [116].

GnRH-a are *de facto* superagonists; indeed, a single injection of 1 µg/kg/bw of buserelin or triptorelin, or other GnRH-a, induces roughly a 10-fold release of LH, compared to the same dosage of native GnRH [99].

When large pharmacologic doses (50–500 µg s.c./day or 300–1200 µg i.m./day) of buserelin is administered for periods greater than 1 to 3 months, a clinical inhibition of gonadotropin release with the subsequent suppression of testosterone, or estradiol, production is obtained.

The high capacity of GnRH-a to induce a super response (at least 10–15 folds higher release of LH) seems due to different factors, among them the binding to the receptors that is markedly higher than that of native GnRH [117,118]. Considering receptor affinity, triptorelin, leuprolide and goserelin bind GnRHR with higher affinity (2 to 14 folds lower K_d, depending on the assay) than GnRH; buserelin shows a K_d from 5 to 70 folds lower respect to native peptide [119–121].

The most common adverse reactions of GnRH-a include headache, loss of libido in patients with prostate cancer and endometriosis, hot flashes, hypermenorrhea, flatulence, impotence, vaginal dryness, back pain, skeletal pain, nasal mucosa irritation, diarrhea, nausea, loss of appetite, insomnia, depression, vertigo and dizziness, palpitation, vaginal dryness, acne, tiredness. Buserelin was found to induce an early,

TABLE 3
Pharmacokinetic data of some GnRH agonists.

Generic Name	Dose	Ab	Cmax	Vd (l)	PB %	T1/2 (days)	CLR (l/hr)	References
Buserelin	200 µg s.c. bolus	1 hr	28 µg/l	44	15	0.04	41*	DB: DB06719 [104,108,110]
Triptorelin	11.25 mg s.c. bolus	4.5 hr	17.17 µg/l	30–33	0	0.1	3.5	EMA: CVMP/382140 DB: DB06825, FDA: 20–715 [105,111]
Goserelin	250 µg s.c. bolus	1 hr	7.79 µg/l	44	20–28	0.2	2	DB: DB00014 FDA: 19–726 S-018 [106,107]
Leuprolide	7.5 mg s.c.	4–5 hr	4.6–212 µg/l (0.1–2 µg/l after 1 month)	27	43–49	0.1	8	DB: DB00007 FDA: 019,943 EMA: 397,961 [109,105,114]

Dose, dose of administration; Ab, absorption peak; Cmax, plasma peak serum concentration; Vd, Volume of distribution; PB, protein binding; T1/2, half-life; CLR, renal clearance.

* Data from pig. DB: DRUGBANK.

transient increase of serum gonadal steroids (testosterone or estradiol) which can lead to the exacerbation of signs and symptoms when used for the treatment of metastatic prostate cancer or endometriosis.

A GnRH-II agonist with a D-Lys⁶ substitution ([D-Lys⁶] GnRH-II) has also been synthesized for experimental use [122] (Table 2).

3.2. GnRH antagonists

The need for GnRH analogs that might block the pituitary–gonadal axis without triggering the unwanted flare-up response led to the design and development of GnRH-ant (Table 2).

GnRH-ant was initially produced to obtain stable analogs that bind GnRHR without its activation. Indeed, they had extensive modifications in the first three AA (the region needed for receptor activation) and a D-AA in position 6 to make them resistant to endopeptidases. The early prototypes underwent a sort of redesign since the first antagonists were unsuitable for clinical applications due to the undesirable induction of a high level of histamine release, with edematogenic effects and other anaphylactic reactions [123]. In the following generation of GnRH-ant such as cetrorelix, ganirelix, abarelix, and degarelix, this side effect was resolved by replacing the D-Arg⁶ with more complex residues (D-ureidoalkyl AA) [124,125].

These compounds share a very low AA similarity with the GnRH decapeptide, and typically, the most used molecules contain Ac-D-Nal-D-Cpa-D-Pal motif in the N-terminal region of the peptide and D-Ala in position 10; moreover, they may also present AA substitutions in positions 5, 6, and 8 [126–128].

GnRH-ant bind human GnRHR with high affinity, in the nM range, that may be up to 20 times higher than the native GnRH [129].

Cetrorelix, degarelix, and ganirelix show the highest efficacy in blocking GnRHR. Abarelix is also highly active, but reports of possible immediate-onset systemic allergic reactions after its administration restricted its marketing [130].

Generally, GnRH-ant have been designed to block the effects of endogenous GnRH on reproductive functions, but different effects were found in peripheral tissues. GnRH analogs showing antagonistic activity on the pituitary GnRHR may behave as agonists in peripheral tissues and exert a GnRH-like antiproliferative activity on tumor cells [84,85,131]. The mechanism of this paradoxical effect is still unclear. However, some hypotheses have been postulated. One of these takes into account the molecular differences (i.e. post-translational modifications like glycosylation and phosphorylation) between central *versus* peripheral GnRHR [132,133]. Another involves the possibility that GnRHR might assume different conformations, according to the cell type in which they are expressed or to the interacting ligand, thus activating different intracellular signaling pathways [22]; this makes the LiSS hypothesis applicable also for GnRH-antagonists.

Cetrorelix was used as a template to develop the first experimental GnRH-II antagonist (Trptorelix-1) by substitution of the AA7 and 8 with Trp and Tyr residues present in GnRH-II [122].

3.2.1. Pharmacokinetic and pharmacodynamic of the GnRH antagonists

The pharmacokinetic parameters of Abarelix, Cetrorelix, Degarelix and Ganirelix are summarized in Table 4.

Cetrorelix and ganirelix show similar characteristics. Both are rapidly absorbed following subcutaneous injection, with a mean absolute bioavailability in healthy female subjects of 85% and 91%, respectively. Cetrorelix was found stable against phase I- and phase II-metabolism; however, only unchanged cetrorelix is detected in urine [134–137].

Cetrorelix and ganirelix dose-dependently inhibit the secretion of LH and FSH from the pituitary gland. The onset of suppression is immediate and is maintained by continuous treatment. The two antagonists are therefore indicated, at 0.25 mg s.c. once daily (q.d.) during the ovarian cycle's early- to mid-follicular phase, for the inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation and for the treatment of infertility. According to what is planned for the development of GnRH-ant, and unlike GnRH-a, which causes an initial increase in gonadotropin levels, these molecules do not cause a *flare-up* effect before premature LH surge inhibition. They are also considered experimental treatments in clinical studies for sex steroid-dependent benign and malignant conditions (e.g., endometriosis, prostate, and ovarian cancer).

Abarelix and degarelix are also similarly indicated for the palliative treatment of advanced prostate cancer. Degarelix is administered s.c. in the abdominal region and forms a depot at the injection site with a slow drug release. It is used at a starting dose of 240 mg followed by a maintenance dose of 80 mg every 28 days; this therapeutic protocol allows to achieve and maintain the suppression of testosterone serum levels under those obtained after medical castration in 97% of patients for at least one year.

Abarelix is also absorbed slowly; following the recommended dose of 100 mg i.m. administration, it shows a mean peak concentration approximately 3 days after the injection. Abarelix quickly drops circulating levels of testosterone (35% at 2 days and 98% at 28 days) [138,139]. Men with symptomatic prostate cancer treated with abarelix showed improvements in pain and disease-related symptoms [140]. However, while degarelix does not seem to induce known systemic allergic reactions [141,142], these have been reported for abarelix [140].

In general, the most common adverse reactions of GnRH-ant include hot flashes, sleep disturbances, breast enlargement or pain, nipple tenderness, diarrhea or constipation, stomach pain, nausea, vomiting, swelling of extremities, nausea, dizziness, headache, fatigue, difficulty urinating, urinary tract infection or upper respiratory tract infection, pain during breathing, feeling short of breath (especially when lying down), rapid heart rate. Sometimes it can induce redness, bruising, itching, pain, erythema, swelling, or induration at the site of injection. An increase in serum levels of transaminases and gamma-glutamyltransferase (GGT) was found after degarelix treatments.

Table 4
Pharmacokinetic data of some GnRH antagonists.

Generic Name	Dose	Ab	Cmax	Vd (l)	PB %	T1/2 (days)	CLR (l/hr)	References
Abarelix	50 ug/kg/day continuous s.c. infusion	28 days	56,1 ug/l	4040	96–99	13.2	0.6	FDA: 021,320 [137]
Cetrorelix	1 mg s.c. bolus	1 hr	20 ug/l	81.2	86	2.61	5.4	FDA: 021,197 [134]
Degarelix	2 mg/kg s.c. bolus	6 hr	330 ug/l	40.9	90	53	9	FDA: 022201; EMA:533396 [135]
Ganirelix	0.25 mg s.c. bolus	1 hr	11.2 ug/l	43.7	81.9	0.53	2.4	FDA: 021,057 [136]

Dose, dose of administration; Ab, absorption peak; Cmax, plasma peak serum concentration; Vd, Volume of distribution; PB, protein binding; T1/2, half-life; CLR, renal clearance.

3.3. Orally active non-peptide GnRH modulators

Therapies employing peptide GnRHR modulators require s.c. or i.m. injection and depot preparations. Therefore, the interest in developing potentially orally active GnRHR agents has always been very high. Oral administration ensures better patient compliance and rapid and dose-dependent control of the HPG axis functions not achievable with non-customized systemic administration GnRH analogs [143,144]. Since the discovery in 1989 of the weak antagonistic activity against GnRHR held by the orally bioavailable antifungal drug ketoconazole, a wide range of non-peptide chemical structures binding the GnRHR were identified [144,145].

In particular, at least three compounds have recently entered clinical use; an uracil-based analogs substituted at positions 1,3,5 and 6, and with a stereocenter in (*R*)-configuration (elagolix), a thieno[2,3-*b*]pyrimidine-2,4-one derivative (relugolix) and a 2,4-dioxo-1,2,3,4-tetrahydrothieno[3,4-*d*]pyrimidine-5-carboxylic acid derivative

(linzagolix) (Fig. 4A).

These orally bioavailable GnRH antagonists bind competitively and specifically to human pituitary GnRHR inducing a stable and reversible suppression of gonadotropin production [146–148]. The results of *in vitro* studies on more than a hundred different membrane receptors, ion channels, enzymes, and transporters, suggest that elagolix, relugolix, and linzagolix have a low risk for off-target pharmacological effects (FDA: 210450; EMA: 13882).

In general, these compounds have been so far used for the management of hormone-dependent diseases; they are well-tolerated and show the same classical side effects of HPG axis blockers as hot flushes and bone mineral decrease, that in case of long-term administration and/or high dosages, can be mitigated through an add-back hormonal therapy (e.g., estradiol and norethindrone acetate) [149–153]. However, this new class of GnRH modulators lacks the histamine-releasing effect shown by the peptide GnRH-ant [154].

Oral non-peptide GnRH-ant thus represents an innovative class of

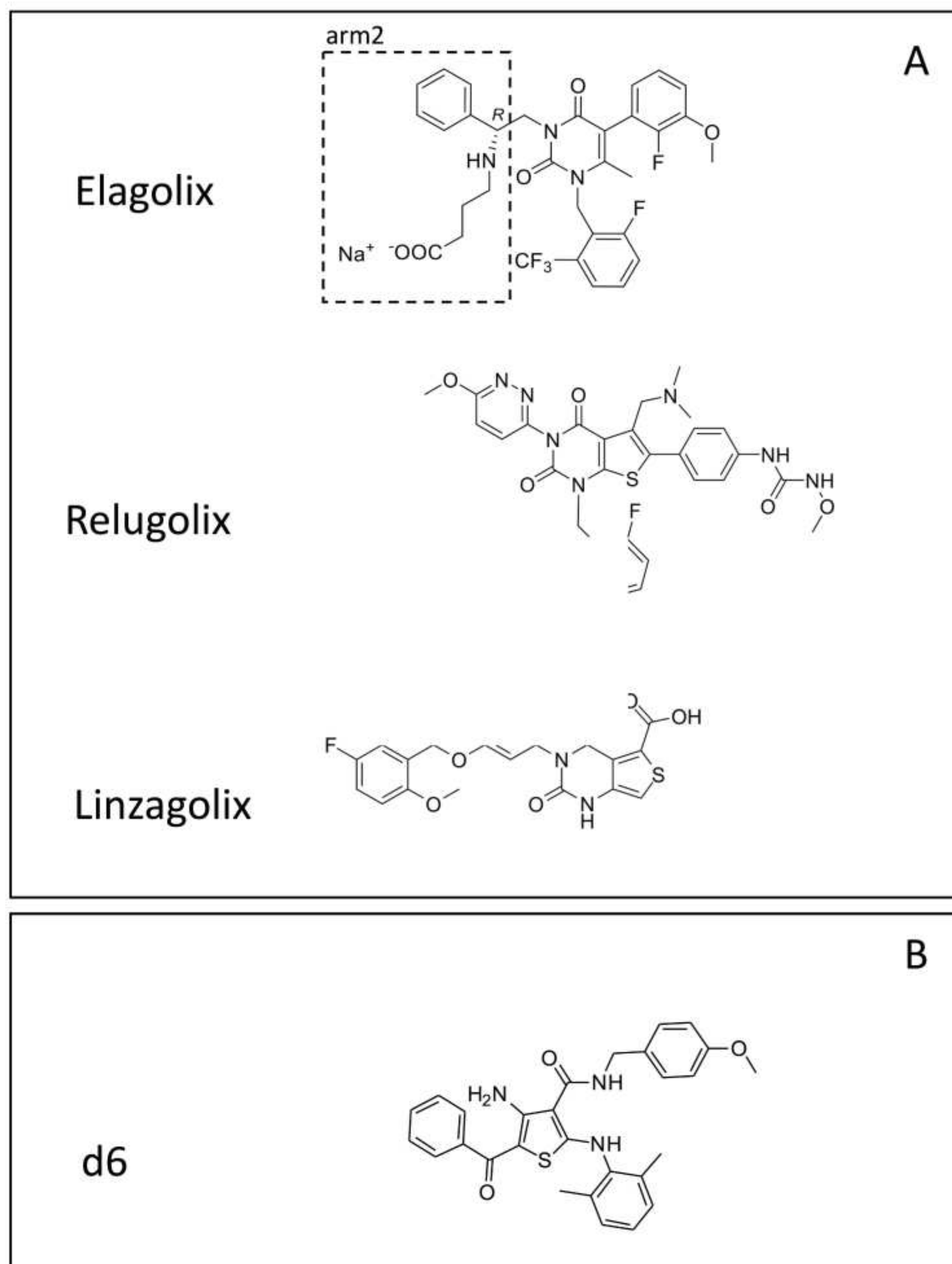


Fig. 4. Non-peptide GnRH modulators. A) Structure of non-peptide antagonists; the region involved in molecule activity (arm2) is indicated; B) structure of the first reported non-peptide GnRH agonist.

drugs for the management of sex-hormone dependent diseases; they are characterized by a fast onset and offset of the therapeutic effect, better patient compliance, and a safety profile superior to peptide GnRH analogs and selective progesterone receptor modulators. Even today, the interest in developing new non-peptide GnRHR modulators with better and better pharmacokinetic and pharmacodynamic properties remains very high. In 2020 [155], Bayer researchers discovered spiroindolines as potent GnRH-ant which, differently by elagolix, relugolix and linzagolix, show similar activities *in vitro* and *in vivo* at both the human and the rat GnRH receptor. This feature will benefit the use of the rat model in the drug optimization process. More recently, was developed a molecular generative model (LS-MolGen) for the design of new orally active GnRHR-ant [156], merging the knowledge about the human GnRHR [157] and the structures of the already-known active compounds.

Of definite interest is the discovery of the first non-peptide GnRHR modulators with agonist activity published in 2022 [157]; the hit compound (6d) contains a tetrasubstituted thiophene core (Fig. 4B) and displayed an EC₅₀ of 1.59 μ M for inositol phosphate accumulation in cells transfected with human GnRHR.

3.3.1. Studies on the interactions of non-peptide modulators with GnRHR

The high species-specificity of non-peptide GnRH antagonists to human GnRH has led to an attempt to elucidate this peculiarity. In the early 2000 s two different groups investigated through systematic mutagenesis experiments the observed dog/human [158] and rat/human [159] GnRHR specificity differences for non-peptide antagonists, never reported for peptide GnRH analogs. Phe³¹³ was found to be of crucial importance in the selectivity difference between dog and human GnRHR. In contrast, the N-terminal domain was imputable for the difference between rat and human GnRHR. These results pointed out that non-peptide ligands do not entirely overlap the binding site of peptide analogs. The subsequent mapping of the human GnRHR residues involved in the binding of peptide GnRH analogs and non-peptide molecules having GnRH antagonistic activity led to the findings that orthosteric non-peptide antagonists occupy distinct partially overlapping subregions within the much larger binding site occupied by peptide analogs but also to the identification of the first example of an allosteric interaction of non-peptide GnRH-ant with GnRHR [160,161]. In 2020, the crystal structure of human GnRHR bound to elagolix was reported [157]; it evidenced the shallow non-peptide antagonist binding site and the interactions required for ligand recognition. The study made it possible to recognize the pyrimidine ring, the benzyl group at position 1 of the uracil moiety, and the phenyl groups of elagolix as a key region to interact with the GnRHR.

It was also found that the residue in position 3 of the uracil core (termed arm2) of elagolix (Fig. 4A) points a large cavity to the extracellular surface of the receptor that allows the recognition of different modifications of this part of the molecule by GnRHR. In addition, by molecular docking simulations, conformational rearrangements of some portions of the binding pocket to better accommodate the more voluminous arm2 of relugolix and sufugolix were observed, suggesting a plasticity of the orthosteric binding pocket concerning different ligands. More recent studies, employing Molecular Dynamics simulations have shown that the binding between non-peptide antagonist and human GnRHR induces a conformational change of TM6 that is moved outward, a well-known activation feature of class A GPCRs [162].

3.3.2. Pharmacokinetic and pharmacodynamic of the non-peptide GnRH antagonists

The non-peptide GnRH-ant bind reversibly with very high affinity to the human GnRHR. Elagolix shows very high affinity to GnRHR (K_d = 54 pM) and inhibits GnRH action *in vitro* with K_i values in the low nM range (1.5 to 2.8 nM) [163].

It is rapidly absorbed from the gastrointestinal tract (relative bioavailability: 52% in human) with an absorption C_{max} occurring at one hour. The compound causes dose-dependent suppression of LH, FSH,

estradiol, and progesterone. At 150 mg q.d. and 200 mg twice a day (b.i.d.) resulted in an ovulation rate of approximately 50% and 32%, respectively. Reduction of estradiol to approximately 12 pg/mL was observed following treatment with 200 mg b.i.d. It has an elimination half-life shorter than other GnRH analogs, ensuring rapid reversibility of its action. This fact is relevant for patients experiencing adverse events since the drug is eliminated within a week. Elagolix is extensively metabolized in the liver, primarily by CYP3A4, to a lesser extent by CYP2D6, and minor metabolism by CYP2C8. 90% of the excretion of elagolix and its metabolites occurs in the feces.

Relugolix binds human and monkey GnRHR with high affinity and shows estimated IC₅₀ value of 0.12–0.15 nM in inhibiting GnRH-a binding [164].

It is rapidly absorbed from the gastrointestinal tract (absolute and relative bioavailability of about 12 and 62%, respectively). In the blood, relugolix is 68.2% to 70.8% bound plasma proteins, primarily to albumin and to a lesser extent to α 1-acid glycoprotein.

In healthy men, after administration of a single 360 mg dose of relugolix, mean serum concentrations of LH and FSH decrease rapidly within 2 h, reaching a nadir of LH concentrations within 24 h. Mean serum testosterone concentrations also decline rapidly within 4 h of administration and reach castration levels (testosterone < 50 ng/dL) within 12–24 h. With multiple doses of 120 mg q.d., testosterone concentrations were maintained consistently at castration levels during treatment. In women, mean estradiol 30.2 pg/mL concentration was observed at 24 h for the 40 mg dose.

Relugolix is mainly metabolized in the liver, primarily by CYP3A4 and, to a lesser extent, by CYP2C8 and other minor unidentified pathways. Approximately 81% of relugolix was excreted in the feces.

Linzagolix also behaves as a highly potent and selective antagonist of the human GnRHR (K_i = 27.4 nM) able to dose-dependently inhibit GnRH-stimulated Ca²⁺ flux with an IC₅₀ value (36.7 nM) comparable to that of the active control cetrorelix (EMA: 266814) [148,165]. A rapid reduction of estradiol to the levels required to treat hormone-dependent diseases such as uterine fibroids and endometriosis is achieved with 100 mg and 200 mg doses. The effects of linzagolix can be quickly reversed, and ovarian activity (estradiol minimal values >40 pg/mL) tends to resume within 2 weeks from the end of treatment [166]. Linzagolix is rapidly absorbed, with a high oral bioavailability (80%) [165]; it strongly binds plasma proteins (greater than 99%) [165], and it is mainly excreted in urine as unchanged drug [165].

Median serum values of linzagolix range between 3250 and 4750 ng/mL after a 100 mg dose and between 6700 ng/mL and 11700 ng/mL after a 200 mg dose [167].

The pharmacokinetic data of these compounds are summarized in Table 5.

The non-peptide GnRH-ant are mainly used for the management of moderate to severe pain associated with endometriosis (elagolix) [168,169], prostate cancer (relugolix) [170], and to uterine fibroids, (elagolix, relugolix, and linzagolix) [165,171]. Currently, clinical phase III trials are ongoing for the treatment of endometriosis-associated pain with relugolix [172] and linzagolix, as announced by ObsEva (CH). More recently, marketing authorization for the use of linzagolix for the treatment of prostate cancer was recommended (EMA:145519). In comparison to elagolix and relugolix, linzagolix could provide more flexible-dose regimens for managing endometriosis-associated pain and uterine fibroids [169].

4. Clinical use of GnRH analogues

4.1. Diseases of the reproductive axis and organs

The characterization of the physiological role of native GnRH has restricted its initial use to ovulation induction in women with reproductive defects due to inadequate peptide secretion. Since early '80, a therapeutic regimen of pulsatile native GnRH has been used to induce

Table 5
Pharmacokinetic data of non-peptide GnRH antagonists.

Generic Name	Dose	Ab	Cmax	Vd (l)	PB %	T1/2 (hr)	CLR (l/hr)	References
Elagolix	150 mg p.o.	1 hr	574 ng/ml	1674	80%	4–6	123	FDA: 210450; EMA:318437
	200 mg p.o.		774 ng/ml	881			144	[163]
Relugolix	80 mg p.o.	1–2 hr	35 ng/ml	19000 ^a	68–71%	36	7.6	FDA: 214621; EMA:145519
								[164]
Linzagolix	100 or 200 mg p.o.	2 hr	145 ug/ml	~11	>99%	15–18	0.5	EMA:266814
								[165,148]

Dose, dose of administration; Ab, absorption peak, Cmax, plasma peak serum concentration; Vd, volume of distribution; PB, protein binding; T1/2, half-life; CL, clearance;

^a derived from the absolute bioavailability study after intravenous administration.

ovulation in women with polycystic ovary syndrome (PCOS), hyperandrogenic anovulation, and late-onset congenital adrenal hyperplasia [95,173]. Since women affected either by CHH (primary GnRH deficiency) or hypothalamic amenorrhea (decreased GnRH release in the presence of an intact hypothalamus) show a higher response rate to the pulsatile administration of GnRH, a GnRH-a therapy should be accurately evaluated in the diagnostic framework of infertility.

The ability of pulsatile GnRH administration to stimulate ovulation led to consideration of its use in ART; a 10 µg GnRH-a pulse can induce the development of multiple (2–5) ovarian follicles [67,174]. GnRH-a induces a more physiological LH and FSH surge and, therefore, a better oocyte quality and a reduced risk of ovarian hyperstimulation syndrome (OHSS), compared to direct ovarian stimulation by administration of gonadotropins (FSH plus human Chorionic Gonadotropin) [175–177]. GnRH-ant has also been indicated in reproductive medicine, gynecology, as well as in ART before gonadotropin stimulation to suppress the endogenous LH surge without an initial stimulatory effect [178].

Another application of GnRH analogs concerns the reversible suppression of the HPG axis. In particular, GnRH-a is considered an effective treatment to restore adult stature, with a concomitant regimen with growth hormone, of girls (up to age 6) with central precocious puberty [179]. The common side effects of such a suppressive regimen may include sex hormone deficiency and menopausal-like symptoms (vaginal dryness and hot flushes). GnRH-a treatment should be generally limited to 6 months due to osteoporosis unless a concomitant estrogen replacement therapy [180].

The HPG-suppressing capacity of GnRH analogs made them applicable for treating several hormone-dependent diseases [181]. Long-term administration of leuprolide or of goserelin is proposed as second-line therapy (after surgery) of endometriosis, a common gynecological condition characterized by the dissemination of endometrial tissue (glands and stroma) to ectopic sites, because, by inducing a hypogonadal state, it deprives ectopic endometrial tissue of estrogen support [182,183].

As stated above, GnRHRs are also expressed in peripheral tissues, mainly in female reproductive tissues, where they may exert autocrine/paracrine actions in both physiological and pathological conditions [132,184]. The finding of locally expressed GnRHRs in human eutopic and ectopic endometrium suggested an additional possible direct action of GnRH-a on endometriotic cells. It has actually been proved that GnRH-a significantly reduces cell proliferation and inflammation, and angiogenesis in endometriotic tissues by decreasing the secretion of pro-mitogenic cytokines [185,186].

4.2. Cancers

Several cancers, including endometrial, ovarian, urinary bladder, and prostate carcinoma, express GnRH and its receptor as part of an autocrine system regulating cell proliferation/metastasis [187]. In addition, breast and non-reproductive cancers (such as pancreatic cancer and glioblastoma) also expressed GnRH/GnRHR system.

The finding of GnRHR in tumor cells has proposed the use of therapeutic strategies based on GnRH analogs for these diseases, and several tumors have been proved to have beneficial effects from the administration of such molecules [187].

Endometrial and ovarian carcinoma are hormone-dependent tumors expressing both estrogen and progesterone (PR) receptors [188,189], in addition, they can benefit from the suppressive GnRH therapy that reduces the production of sex steroids. Nevertheless, GnRH-a (e.g., triptorelin, leuporelin, goserelin, buserelin) were found to exert a significant antiproliferative effect on endometrial and ovarian cancer cell lines [131,190,191]. The proliferation of such cells is also inhibited by GnRH-ant [84,131] confirming that these compounds may behave as agonists at the level of tumoral GnRHRs. After discovering the expression of GnRH-II in the reproductive tract, its possible role in the control of tumor growth was investigated. GnRH-II analogs have also been found to exert a significant antiproliferative effect on human ovarian and endometrial cells by activating the classical form (type I) of the GnRHR [192,193].

Prostate cancer is the most aggressive tumor and the second most frequent cause of tumor-related deaths among men in western countries [194]. The growth of cancer may be dependent on androgens; therefore, patients may benefit from androgen-deprivation therapy (i.e., chemical castration) with GnRH-a, often in combination with drugs altering the androgen receptor signaling (e.g., antiandrogens, inhibitors of androgen synthesis). Several pieces of evidence from *in vivo* studies indicate that GnRH-a may exert significant antiproliferative/proapoptotic, anti-metastatic, and antiangiogenic activities on prostate cancer cells [195]. Some clinical trials have analyzed the use of GnRH-ant to induce medical castration in men with advanced or metastatic prostate cancer; the results do not report significant overall survival or cancer-specific survival by comparing GnRH-ant to standard androgen suppression therapy [196,197]. Therefore, the therapy with GnRH-a remains the preferred option for the treatment of prostate cancer.

Suppressive GnRH-a therapy, resulting in decreased estrogen circulating levels, has also been successfully used to reduce the growth and recurrence of hormonal responsive breast tumors. Once more, GnRH-a may also exert direct action on breast cancer, reducing cell growth and invasion. GnRH-a (triptorelin, buserelin and goserelin) and antagonist (cetorelix), non-peptide GnRH-ant, as well as GnRH-II analogs and cytotoxic analogs of GnRH, have been tested as novel adjuvant therapies in breast cancer and the data suggest that the GnRH/GnRHR system may be a promising target for pharmacological approach in the treatment of advanced states of such tumor [198]. An interesting aspect lies in the observation that GnRH-II-ant may have a direct effect on malignant tumors, inducing cell death via the apoptotic process [199]. On the other hand, in cellular models of human breast carcinoma such as MCF-7, the administration of [Ac-D2Nal1, D-4Cpa2, D-3Pal3,6, Leu8, D-Ala10]-GnRH-II induced the activation of apoptosis and significant inhibition of tumor growth [193,198].

The effects of GnRH analogs in pancreatic tumors and glioblastoma are limited to preclinical investigation [200,201].

The higher expression of GnRHR in cancer cells than in other tissues,

also raised the hypothesis to use GnRH analogs as carriers for targeted chemotherapy of tumors [202–204]. This was achieved by linking a traditional anticancer drug to a GnRH analog and designed to increase the selectivity of cytotoxic drugs to GnRHR expressing cells, decreasing the peripheral toxicity of chemotherapy [205]. After the binding to GnRHR, the bioconjugates are internalized by endocytosis and before being degraded in lysosomes they release the free anticancer drug that can exert its cytotoxic effect. Currently, the most used compound is the analog [D-Lys⁶]-GnRH linked to doxorubicin through the ϵ -amino group of D-Lys (AEZS-108). Clinical trials demonstrate that AESZ-108 exerts significant anti-tumor activity, with low toxicity, in women with advanced or recurrent GnRHR positive gynecological cancers [202].

In conclusion, the data on the effects of GnRH analogs in tumors are promising; new evidence also suggests that GnRH-based cytotoxic bioconjugates offer a new strategy in treating GnRHR expressing tumors, even in their aggressive phase.

4.3. Fertility preservation in female patients undergoing chemotherapy

Chemotherapy and radiation therapy are still the main treatments for many cancer diseases. When applied to young women, chemotherapeutic drugs may cause apoptotic death in oocytes. On the other hand, radiotherapy also induces ovarian failure and endometrial insufficiency, resulting in adverse reproduction outcomes. Exposure to chemotherapeutic drugs kills the developing ovarian follicles, thus accelerating primordial/primary follicles activation, resulting in a “burnout” effect [206] with consequent exhaustion of the follicles. Effective strategies to minimize gonadal damage, and preserve ovarian function and fertility, have become of increasing interest [207,208].

Existing strategies, like ovarian tissue cryopreservation, oocyte cryopreservation, *in vitro* fertilization followed by cryopreservation of the embryos, may require the postponement of chemotherapy for several days, do not guarantee future fertility and the opportunities for conception largely depend on the number of cryopreserved oocytes/embryos [207,209].

A pharmacological approach (*ferto-protective adjuvant therapy*) has been introduced to minimize the gonadotoxic effects of chemotherapy and prevent premature ovarian failure. This approach is based on the administration of GnRH-a during or before chemotherapy [210,211]. GnRH-a reduces the activity of the HPG axis and the activation of primordial ovarian follicles, preventing burnout and ovarian failure. In addition, they may decrease ovarian perfusion, may exert a direct antitumor activity (based on the tumor expression of GnRHR), and protect the ovarian germinative stem cells [208,210]. Nevertheless, the efficacy of GnRH-a in preserving ovarian function and fertility in young patients undergoing chemotherapy needs definitive confirmations [211].

4.4. Possible applications in neurological diseases

Recent observations indicated a wide expression of GnRHR/GnRH system in the CNS, mainly in the hippocampus and amygdala, two regions implicated in cognitive and sexual behavior. In these areas, GnRH, acting as a neuromodulator, might play specific functions not depicted yet, but that may be correlated to some neurological disease.

For instance, more than 50 million people suffer from dementia, and two-thirds of these are diagnosed as Alzheimer's disease (AD).

The higher prevalence of Alzheimer's disease in women than in men [212] was linked to a decrease of circulating levels of estrogens, which, as widely reported in rodents and *in vitro* models, may exert neuroprotective effects [213,214]. Likely, during menopause (physiological or surgically induced), there is a dramatic decrease in estrogen levels in the brain, making neurons more vulnerable to degeneration. It is now well known that hippocampal synaptic spine density depends on estradiol and testosterone, either originating from the gonads or locally synthesized in the hippocampus. Women taking estrogen supplements or that

underwent menopause later in life had a significantly decreased risk of developing AD [213]. On the other hand, the observed greatest increase of gonadotropin levels due to the lack of estrogen negative feedback, is suggestive to the exposure of aged women brain to higher levels of GnRH than man counterpart and GnRH was found to affect rat hippocampal dendritic spine density changes occurring during the estrus cycle through the local regulation of aromatase activity and therefore to estradiol synthesis [215].

However, in estrogen-depleted animal models, the administration of GnRH-a leuprolide acetate induces a cognitive improvement, such as the Morris water maze and Y-maze tests.

These effects were not found to be due to modification of local estrogen production and provide evidence that estrogen- and leuprolide-induced cognitive enhancement may be driven by independent molecular mechanisms [216]. However, further studies indicated that leuprolide does not exert significant effects on AD outcomes in three clinical trials so far conducted (NCT00076440, NCT00063310, NCT00231946,); in a fourth ongoing study, scheduled to be completed in 2026, GnRH-a and cholinesterase inhibitors are administered in combination (NCT03649724), assuming that better results could be obtained than a single administration.

Leuprolide has also been recently proposed for other neurological diseases. For instance, long-term treatment with the leuprolide acetate (up to 84 months trial UMIN000000474) shows to delay the functional decline and suppress the incidence of pneumonia and death in individuals affected by spinal and bulbar muscular atrophy (SBMA) [217], a genetic X-linked recessive neuromuscular disease [218]. Further trials found that this GnRH-a may be effective in improving the swallowing capacity of the patients [219] and another trial on leuprolide in SBMA (NCT03555578) is ongoing.

Spinal cord injury (SCI) could also benefit from GnRH-a treatment; experimental evidence obtained using a compression model of SCI in rats, indicate that 5 weeks of administration of leuprolide induced a higher locomotor activity recovery (up to 38%) than untreated rats (7%) and a greater preservation of white and gray matter in the spinal cord of the injured rat [220]. A similar preclinical study, performed in SCI ovariectomized rats, indicated that leuprolide was able to induce a significant improvement of the neurogenic colon [221]. In other studies, patients with chronic SCI who underwent leuprolide administration once a month for six months have shown an improvement of sensory scores, motor activity, and level of independence [222].

Finally, the treatment of experimental autoimmune encephalomyelitis rats with leuprolide was found to exert a neurotrophic action with a decreased severity of clinical signs of locomotion, a greater body weight gain and expression of disease-affected proteins, suggesting the potential therapeutic application in neurological inflammatory diseases such as multiple sclerosis (MS) [223]. In contrast, it has also been reported that in MS affected women underwent to ART, the use of GnRH analogs seems to correlate with an increase in disease activity [224]; moreover, neurological deficits suggesting MS exacerbation were reported in a case of a woman treated with leuprolide for uterine fibroids [225].

Considering the involvement of estrogens in neurological disorders [226] and that pregnancy and delivery may affect MS activity [227], further studies are necessary to elucidate how GnRH analog-induced hormonal changes might affect MS course.

5. Conclusions

GnRH analogs (agonists and antagonists) are used in consolidated therapeutic protocols to restore GnRH insufficiency or suppress fertility in men and women. GnRH analogs have since emerged as a powerful tool for treating several diseases not only related to the reproductive organs but also cancer and neurological disorders. Although many efforts are directed toward developing new non-peptide analogs to ensure better patient compliance, more in-depth studies and further

characterization of the new pharmacological properties and delivery protocols of the GnRH analogs will enable increasingly efficient use of these drugs.

CRedit authorship contribution statement

Lavinia Casati: Conceptualization, Writing – original draft, Writing – review & editing. **Samuele Ciceri:** Conceptualization, Writing – original draft. **Roberto Maggi:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Daniele Bottai:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] A.V. Schally, A. Arimura, Y. Baba, R.M. Nair, H. Matsuo, T.W. Redding, L. Debeljuk, Isolation and properties of the FSH and LH-releasing hormone, *Biochem. Biophys. Res. Commun.* 43 (2) (1971) 393–399.
- [2] R. Maggi, A.M. Cariboni, M.M. Marelli, R.M. Moretti, V. Andr , M. Marzagalli, P. Limonta, GnRH and GnRH receptors in the pathophysiology of the human female reproductive system, *Hum. Reprod. Update* 22 (3) (2016) 358–381.
- [3] P. Limonta, M.M. Marelli, R. Moretti, M. Marzagalli, F. Fontana, R. Maggi, GnRH in the Human Female Reproductive Axis, *Vitam and Horm* 107 (2018) 27–66.
- [4] P. Le Tissier, P. Campos, C. Lafont, N. Roman , D.J. Hodson, P. Mollard, An updated view of hypothalamic-vascular-pituitary unit function and plasticity, *Nat. Rev. Endocrinol.* 13 (5) (2017) 257–267.
- [5] R.P. Millar, Z.L. Lu, A.J. Pawson, C.A. Flanagan, K. Morgan, S.R. Maudsley, Gonadotropin-releasing hormone receptors, *Endocr. Rev.* 25 (2) (2004) 235–275.
- [6] N.M. Aubead, Role of sex hormone in human body, in: C. Marsh (Ed.), *Reproductive May 5th, 2021*, IntechOpen, Doi: 10.5772/intechopen.91499.
- [7] N.D. Shaw, S.N. Histed, S.S. Srouji, J. Yang, H. Lee, J.E. Hall, Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women, *J. Clin. Endocrinol. Metab.* 95 (4) (2010) 1955–1961.
- [8] M. Bonomi, D.V. Libri, F. Guizzardi, E. Guarducci, E. Maiolo, E. Pignatti, R. Asci, L. Persani, Idiopathic Central Hypogonadism Study Group of the Italian Societies of Endocrinology and Pediatric Endocrinology and Diabetes, New understandings of the genetic basis of isolated idiopathic central hypogonadism, *Asian J Androl* 14 (1) (2012) 49–56.
- [9] P.H. Seeburg, A.J. Mason, T.A. Stewart, K. Nikolics, The mammalian GnRH gene and its pivotal role in reproduction, *Recent Prog. Horm. Res.* 43 (1987) 69–98.
- [10] R.P. Millar, GnRHs and GnRH receptors, *Anim. Reprod. Sci.* 88 (1–2) (2005) 5–28.
- [11] B.S. Abel, N.D. Shaw, J.M. Brown, J.M. Adams, T. Alati, K.A. Martin, N. Pitteloud, S.B. Seminara, L. Plummer, D. Pignatelli, W.F. Crowley Jr., C.K. Welt, J.E. Hall, Responsiveness to a physiological regimen of GnRH therapy and relation to genotype in women with isolated hypogonadotropic hypogonadism, *J. Clin. Endocrinol. Metab.* 98 (2) (2013) E206–E216.
- [12] C. Duan, J. Allard, Gonadotropin-releasing hormone neuron development in vertebrates, *Gen. Comp. Endocrinol.* 292 (2020), 113465.
- [13] G.J. Roch, E.R. Busby, N.M. Sherwood, GnRH receptors and peptides: skating backward, *Gen. Comp. Endocrinol.* 209 (2014) 118–134.
- [14] P.C. Leung, C.K. Cheng, X.M. Zhu, Multi-factorial role of GnRH-I and GnRH-II in the human ovary, *Mol. Cell. Endocrinol.* 202 (1–2) (2003) 145–153.
- [15] W.H. Yu, S. Karanth, A. Walczewska, S.A. Sower, S.M. McCann, A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat, *PNAS* 94 (17) (1997) 9499–9503.
- [16] V.S. Latimer, S.M. Rodrigues, V.T. Garyfallou, S.G. Kohama, R.B. White, R. D. Fernald, H.F. Urbanski, Two molecular forms of gonadotropin-releasing hormone (GnRH-I and GnRH-II) are expressed by two separate populations of cells in the rhesus macaque hypothalamus, *Brain Res. Mol. Brain Res.* 75 (2) (2000) 287–292.
- [17] A.S. Kauffman, Emerging functions of gonadotropin-releasing hormone II in mammalian physiology and behaviour, *J. Neuroendocrinol.* 16 (9) (2004) 794–806.
- [18] V.S. Densmore, H.F. Urbanski, Relative effect of gonadotropin-releasing hormone (GnRH)-I and GnRH-II on gonadotropin release, *J. Clin. Endocrinol. Metab.* 88 (5) (2003) 2126–2134.
- [19] H.F. Urbanski, Differential roles of GnRH-I and GnRH-II neurons in the control of the primate reproductive axis, *Front Endocrinol (Lausanne)* 3 (2012) 20.
- [20] K.W. Cheon, H.S. Lee, I.S. Parhar, I.S. Kang, Expression of the second isoform of gonadotropin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle, *Mol. Hum. Reprod.* 7 (5) (2001) 447–452.
- [21] I.S. Hong, A.P. Cheung, P.C. Leung, Gonadotropin-releasing hormones I and II induce apoptosis in human granulosa cells, *J. Clin. Endocrinol. Metab.* 93 (8) (2008) 3179–3185.
- [22] R.P. Millar, A.J. Pawson, K. Morgan, E.F. Rissman, Z.L. Lu, Diversity of actions of GnRHs mediated by ligand-induced selective signaling, *Front. Neuroendocrinol.* 29 (1) (2008) 17–35.
- [23] S.A. Sower, Y.C. Chiang, S. Lovas, J.M. Conlon, Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain, *Endocrinology* 132 (3) (1993) 1125–1131.
- [24] K.L. Deragon, S.A. Sower, Effects of lamprey gonadotropin-releasing hormone-III on steroidogenesis and spermiogenesis in male sea lampreys, *Gen. Comp. Endocrinol.* 95 (3) (1994) 363–367.
- [25] M. Kovacs, J. Seprodi, M. Koppan, J.E. Horvath, B. Vincze, I. Teplan, B. Flerko, Lamprey gonadotropin hormone-releasing hormone-III has no selective follicle-stimulating hormone-releasing effect in rats, *J. Neuroendocrinol.* 14 (8) (2002) 647–655.
- [26] N.C. Fan, E.B. Jeung, C. Peng, J.I. Olofsson, J. Krisinger, P.C. Leung, The human gonadotropin-releasing hormone (GnRH) receptor gene: cloning, genomic organization and chromosomal assignment, *Mol. Cell. Endocrinol.* 103 (1–2) (1994) R1–R6.
- [27] S.S. Kakar, M.T. Malik, S.J. Winters, W. Mazhawidza, Gonadotropin-releasing hormone receptors: structure, expression, and signaling transduction, *Vitam. Horm.* 69 (2004) 151–207.
- [28] S.S. Ferguson, Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling, *Pharmacol. Rev.* 53 (1) (2001) 1–24.
- [29] J.N. Hislop, M.T. Madziva, H.M. Everest, T. Harding, J.B. Uney, G.B. Willars, R. P. Millar, B.E. Troskie, J.S. Davidson, C.A. McArdle, Desensitization and internalization of human and xenopus gonadotropin-releasing hormone receptors expressed in alphaT4 pituitary cells using recombinant adenovirus, *Endocrinology* 141 (12) (2000) 4564–4575.
- [30] A. Heding, M. Vrecl, J. Bogerd, A. McGregor, R. Sellar, P.L. Taylor, K.A. Eidne, Gonadotropin-releasing hormone receptors with intracellular carboxyl-terminal tails undergo acute desensitization of total inositol phosphate production and exhibit accelerated internalization kinetics, *J. Biol. Chem.* 273 (19) (1998) 11472–11477.
- [31] A. Heding, M. Vrecl, A.C. Hanyaloglu, R. Sellar, P.L. Taylor, K.A. Eidne, The rat gonadotropin-releasing hormone receptor internalizes via a beta-arrestin-independent, but dynamin-dependent, pathway: addition of a carboxyl-terminal tail confers beta-arrestin dependency, *Endocrinology* 141 (1) (2000) 299–306.
- [32] M. Vrecl, L. Anderson, A. Hanyaloglu, A.M. McGregor, A.D. Groarke, G. Milligan, P.L. Taylor, K.A. Eidne, Agonist-induced endocytosis and recycling of the gonadotropin-releasing hormone receptor: effect of beta-arrestin on internalization kinetics, *Mol. Endocrinol.* 12 (12) (1998) 1818–1829.
- [33] G.B. Willars, A. Heding, M. Vrecl, R. Sellar, M. Blomenr hr, S.R. Nahorski, K. A. Eidne, Lack of a C-terminal tail in the mammalian gonadotropin-releasing hormone receptor confers resistance to agonist-dependent phosphorylation and rapid desensitization, *J. Biol. Chem.* 274 (42) (1999) 30146–30153.
- [34] G.A. Stamatiades, C. Toufaily, H.K. Kim, X. Zhou, I.R. Thompson, R.S. Carroll, M. Chen, L.S. Weinstein, S. Offermanns, U. Boehm, D.J. Bernard, U.B. Kaiser, Deletion of Gαq/11 or Gas Proteins in Gonadotropes Differentially Affects Gonadotropin Production and Secretion in Mice, *Endocrinology* 163 (2) (2022) bqab247.
- [35] F. Liu, I. Usui, L.G. Evans, D.A. Austin, P.L. Mellon, J.M. Olefsky, N.J. Webster, Involvement of both G(q/11) and G(s) proteins in gonadotropin-releasing hormone receptor-mediated signaling in L beta T2 cells, *J. Biol. Chem.* 277 (35) (2002) 32099–32108.
- [36] S. Kraus, Z. Naor, R. Seger, Intracellular signaling pathways mediated by the gonadotropin-releasing hormone (GnRH) receptor, *Arch. Med. Res.* 32 (6) (2001) 499–509.
- [37] L. Jennes, W.E. Stumpf, P.M. Conn, Intracellular pathways of electron-opaque gonadotropin-releasing hormone derivatives bound by cultured gonadotropes, *Endocrinology* 113 (5) (1983) 1683–1689.
- [38] L. Jennes, D. Coy, P.M. Conn, Receptor-mediated uptake of GnRH agonist and antagonists by cultured gonadotropes: evidence for differential intracellular routing, *Peptides* 7 (3) (1986) 459–463.
- [39] P.M. Conn, D.D. Staley, T. Yasumoto, W.R. Huckle, J.A. Janovick, Homologous desensitization with gonadotropin-releasing hormone (GnRH) also diminishes gonadotrope responsiveness to maitotoxin: a role for the GnRH receptor-regulated calcium ion channel in mediation of cellular desensitization, *Mol. Endocrinol.* 1 (2) (1987) 154–159.
- [40] J.S. Davidson, I.K. Wakefield, R.P. Millar, Absence of rapid desensitization of the mouse gonadotropin-releasing hormone receptor, *Biochem. J* 300 (Pt 2) (1994) 299–302.
- [41] C.A. McArdle, J. Franklin, L. Green, J.N. Hislop, Signalling, cycling and desensitisation of gonadotropin-releasing hormone receptors, *J. Endocrinol.* 173 (1) (2002) 1–11.

- [42] G.B. Willars, J.E. Royall, S.R. Nahorski, F. El-Gehani, H. Everest, C.A. McArdle, Rapid down-regulation of the type I inositol 1,4,5-trisphosphate receptor and desensitization of gonadotropin-releasing hormone-mediated Ca^{2+} responses in alpha T3-1 gonadotropes, *J. Biol. Chem.* 276 (5) (2001) 3123–3129.
- [43] M. Murase, T. Uemura, M. Gao, M. Inada, T. Funabashi, F. Hirahara, GnRH antagonist-induced down-regulation of the mRNA expression of pituitary receptors: comparisons with GnRH agonist effects, *Endocr. J.* 52 (1) (2005) 131–137.
- [44] M.M. Janjic, R.M. Prévêde, P.A. Fletcher, A. Sherman, K. Smiljanic, D. Abebe, I. Bjelobaba, S.S. Stojilkovic, Divergent expression patterns of pituitary gonadotropin subunit and GnRH receptor genes to continuous GnRH in vitro and in vivo, *Sci. Rep.* 9 (1) (2019) 20098.
- [45] L.F. Silveira, G.S. MacColl, P.M. Bouloux, Hypogonadotropic hypogonadism, *Semin. Reprod. Med.* 20 (4) (2002) 327–338.
- [46] R. Millar, S. Lowe, D. Conklin, A. Pawson, S. Maudsley, B. Troskie, T. Ott, M. Millar, G. Lincoln, R. Sellar, B. Faurholm, G. Scobie, R. Kuestner, E. Terasawa, A. Katz, A novel mammalian receptor for the evolutionarily conserved type II GnRH, *PNAS* 98 (17) (2001) 9636–9641.
- [47] J.D. Neill, L.W. Duck, J.C. Sellers, L.C. Musgrove, A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH II in primates, *Biochem. Biophys. Res. Commun.* 282 (4) (2001) 1012–1018.
- [48] J.D. Neill, L.C. Musgrove, L.W. Duck, Newly recognized GnRH receptors: function and relative role, *Trends Endocrinol. Metab.* 15 (8) (2004) 383–392.
- [49] M. Montagnani Marelli, R.M. Moretti, S. Mai, J. Januszkiewicz-Caulier, M. Motta, P. Limonta, Type I gonadotropin-releasing hormone receptor mediates the antiproliferative effects of GnRH-II on prostate cancer cells, *J. Clin. Endocrinol. Metab.* 94 (5) (2009) 1761–1767.
- [50] M. Montagnani Marelli, M. Manea, R.M. Moretti, M. Marzagalli, P. Limonta, Oxime bond-linked daunorubicin-GnRH-III bioconjugates exert antitumor activity in castration-resistant prostate cancer cells via the type I GnRH receptor, *Int. J. Oncol.* 46 (1) (2015) 243–253.
- [51] H.M. Wu, H.Y. Huang, C.L. Lee, Y.K. Soong, P.C. Leung, H.S. Wang, Gonadotropin-Releasing Hormone Type II (GnRH-II) Agonist Regulates the Motility of Human Decidual Endometrial Stromal Cells: Possible Effect on Embryo Implantation and Pregnancy, *Biol. Reprod.* 92 (4) (2015) 98.
- [52] R.R. Katreddi, P.E. Forni, Mechanisms underlying pre- and postnatal development of the vomeronasal organ, *Cell. Mol. Life Sci.* 78 (12) (2021) 5069–5082.
- [53] F. Casoni, S.A. Malone, M. Belle, F. Luzzati, F. Collier, C. Allet, E. Hrabovszky, S. Rasika, V. Prevot, A. Chédotal, P. Giacobini, Development of the neurons controlling fertility in humans: new insights from 3D imaging and transparent fetal brains, *Development* 143 (21) (2016) 3969–3981.
- [54] D.J. Spergel, Modulation of Gonadotropin-Releasing Hormone Neuron Activity and Secretion in Mice by Non-peptide Neurotransmitters, Gasotransmitters, and Gliotransmitters, *Front Endocrinol (Lausanne)* 10 (2019) 329.
- [55] C.F. Aylwin, K. Vigh-Conrad, A. Lomniczi, The Emerging Role of Chromatin Remodeling Factors in Female Pubertal Development, *Neuroendocrinology* 109 (3) (2019) 208–217.
- [56] M. Schwanzel-Fukuda, D. Bick, D.W. Pfaff, Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome, *Brain Res. Mol. Brain Res.* 6 (4) (1989) 311–326.
- [57] B. Franco, S. Guioli, A. Pragliola, B. Incerti, B. Bardoni, R. Tonlorenzi, R. Carrozzo, E. Maestrini, M. Pieretti, P. Taillon-Miller, C.J. Brown, H.F. Willard, C. Lawrence, M. Graziella Persico, G. Camerino, A. Ballabio, A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules, *Nature* 353 (6344) (1991) 529–536.
- [58] D. Lopez-Rodriguez, D. Franssen, J. Bakker, A. Lomniczi, A.S. Parent, Cellular and molecular features of EDC exposure: consequences for the GnRH network, *Nat. Rev. Endocrinol.* 17 (2) (2021) 83–96.
- [59] L.F. Silveira, E.B. Trarbach, A.C. Latronico, Genetics basis for GnRH-dependent pubertal disorders in humans, *Mol. Cell. Endocrinol.* 324 (1–2) (2010) 30–38.
- [60] E.L. Stevenson, K.M. Corella, W.C. Chung, Ontogenesis of gonadotropin-releasing hormone neurons: a model for hypothalamic neuroendocrine cell development, *Front Endocrinol (Lausanne)* 4 (2013) 89.
- [61] S.P. Kalra, Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge, *Endocr. Rev.* 14 (5) (1993) 507–538.
- [62] J.C. Marshall, A.C. Dalkin, D.J. Haisenleder, S.J. Paul, G.A. Ortolano, R.P. Kelch, Gonadotropin-releasing hormone pulses: regulators of gonadotropin synthesis and ovulatory cycles, *Recent Prog. Horm. Res.* 47 (1991), 155–87; discussion 188–9.
- [63] R. Tsutsumi, N.J. Webster, GnRH pulsatility, the pituitary response and reproductive dysfunction, *Endocr. J.* 56 (6) (2009) 729–737.
- [64] E. Hrabovszky, Z. Liposits, Afferent neuronal control of type-I gonadotropin releasing hormone neurons in the human, *Front Endocrinol (Lausanne)* 4 (2013) 130.
- [65] L.V. DePaolo, R.A. King, A.J. Carrillo, In vivo and in vitro examination of an autoregulatory mechanism for luteinizing hormone-releasing hormone, *Endocrinology* 120 (1) (1987) 272–279.
- [66] F.J. Hayes, W.F. Crowley, Gonadotropin pulsations across development, *Horm. Res.* 49 (3–4) (1998) 163–168.
- [67] K. Martin, N. Santoro, J. Hall, M. Filicori, M. Wierman, W.F. Crowley, Clinical review 15: Management of ovulatory disorders with pulsatile gonadotropin-releasing hormone, *J. Clin. Endocrinol. Metab.* 71 (5) (1990) 1081A–A.
- [68] P.E. Belchetz, T.M. Plant, Y. Nakai, E.J. Keogh, E. Knobil, Hypophysial responses to continuous and intermittent delivery of hypophyseal gonadotropin-releasing hormone, *Science* 202 (4368) (1978) 631–633.
- [69] E. Loumaye, K.J. Catt, Homologous regulation of gonadotropin-releasing hormone receptors in cultured pituitary cells, *Science* 215 (4535) (1982) 983–985.
- [70] K.W. Cheng, P.S. Nathwani, P.C. Leung, Regulation of human gonadotropin-releasing hormone receptor gene expression in placental cells, *Endocrinology* 141 (7) (2000) 2340–2349.
- [71] R. Tsutsumi, D. Mistry, N.J. Webster, Signaling responses to pulsatile gonadotropin-releasing hormone in LbetaT2 gonadotrope cells, *J. Biol. Chem.* 285 (26) (2010) 20262–20272.
- [72] J. Fortin, P. Lamba, Y. Wang, D.J. Bernard, Conservation of mechanisms mediating gonadotrophin-releasing hormone 1 stimulation of human luteinizing hormone beta subunit transcription, *Mol. Hum. Reprod.* 15 (2) (2009) 77–87.
- [73] S.M. McGillivray, V.G. Thackray, D. Coss, P.L. Mellon, Activin and glucocorticoids synergistically activate follicle-stimulating hormone beta-subunit gene expression in the immortalized LbetaT2 gonadotrope cell line, *Endocrinology* 148 (2) (2007) 762–773.
- [74] A.A. Dwyer, F.J. Hayes, L. Plummer, N. Pitteloud, W.F. Crowley Jr., The long-term clinical follow-up and natural history of men with adult-onset idiopathic hypogonadotropic hypogonadism, *J. Clin. Endocrinol. Metab.* 95 (9) (2010) 4235–4243.
- [75] T.S. Han, P.M. Bouloux, What is the optimal therapy for young males with hypogonadotropic hypogonadism? *Clin. Endocrinol.* 72 (6) (2010) 731–737.
- [76] C.A. McArdle, Gonadotropin-releasing hormone receptor signaling: biased and unbiased, *Mini Rev. Med. Chem.* 12 (9) (2012) 841–850.
- [77] L.S. Lin, V.J. Roberts, S.S. Yen, Expression of human gonadotropin-releasing hormone receptor gene in the placenta and its functional relationship to human chorionic gonadotropin secretion, *J. Clin. Endocrinol. Metab.* 80 (2) (1995) 580–585.
- [78] G.S. Harrison, M.E. Wierman, T.M. Nett, L.M. Glode, Gonadotropin-releasing hormone and its receptor in normal and malignant cells, *Endocr. Relat. Cancer* 11 (4) (2004) 725–748.
- [79] P. Limonta, D. Dondi, R.M. Moretti, R. Maggi, M. Motta, Antiproliferative effects of luteinizing hormone-releasing hormone agonists on the human prostatic cancer cell line LNCaP, *J. Clin. Endocrinol. Metab.* 75 (1) (1992) 207–212.
- [80] D. Dondi, P. Limonta, R.M. Moretti, M.M. Marelli, E. Garattini, M. Motta, Antiproliferative effects of luteinizing hormone-releasing hormone (LHRH) agonists on human androgen-independent prostate cancer cell line DU 145: evidence for an autocrine-inhibitory LHRH loop, *Cancer Res.* 54 (15) (1994) 4091–4095.
- [81] A. Imai, T. Ohno, K. Iida, T. Fuseya, T. Furui, T. Tamaya, Presence of gonadotropin-releasing hormone receptor and its messenger ribonucleic acid in endometrial carcinoma and endometrium, *Gynecol. Oncol.* 55 (1) (1994) 144–148.
- [82] G. Irmer, C. Burger, R. Muller, O. Ortmann, U. Peter, S.S. Kakar, J.D. Neill, K. D. Schulz, G. Emons, Expression of the messenger RNAs for luteinizing hormone-releasing hormone (LHRH) and its receptor in human ovarian epithelial carcinoma, *Cancer Res.* 55 (4) (1995) 817–822.
- [83] K. Walters, I.N. Wegorzewska, Y.P. Chin, M.G. Parikh, T.J. Wu, Luteinizing hormone-releasing hormone I (LHRH-I) and its metabolite in peripheral tissues, *Exp. Biol. Med.* (Maywood) 233 (2) (2008) 123–130.
- [84] D. Kleinman, A. Douvdevani, A.V. Schally, J. Levy, Y. Sharoni, Direct growth inhibition of human endometrial cancer cells by the gonadotropin-releasing hormone antagonist SB-75: role of apoptosis, *Am. J. Obstet. Gynecol.* 170 (1 Pt 1) (1994) 96–102.
- [85] T. Yano, J. Pinski, G. Halmos, K. Szepeshazi, K. Groot, A.V. Schally, Inhibition of growth of OV-1063 human epithelial ovarian cancer xenografts in nude mice by treatment with luteinizing hormone-releasing hormone antagonist SB-75, *PNAS* 91 (15) (1994) 7090–7094.
- [86] A.R. Gunthert, C. Grundker, K. Hollmann, G. Emons, Luteinizing hormone-releasing hormone induces JunD-DNA binding and extends cell cycle in human ovarian cancer cells, *Biochem. Biophys. Res. Commun.* 294 (1) (2002) 11–15.
- [87] K.Y. Kim, K.C. Choi, S.H. Park, C.S. Chou, N. Auersperg, P.C. Leung, Type II gonadotropin-releasing hormone stimulates p38 mitogen-activated protein kinase and apoptosis in ovarian cancer cells, *J. Clin. Endocrinol. Metab.* 89 (6) (2004) 3020–3026.
- [88] W.F. Crowley, J.W. McArthur, Simulation of the normal menstrual cycle in Kallmann's syndrome by pulsatile administration of luteinizing hormone-releasing hormone (LHRH), *J. Clin. Endocrinol. Metab.* 51 (1) (1980) 173–175.
- [89] D.S. Miller, R.R. Reid, N.S. Cetel, R.W. Rebar, S.S. Yen, Pulsatile administration of low-dose gonadotropin-releasing hormone, Ovulation and pregnancy in women with hypothalamic amenorrhea, *JAMA* 250 (21) (1983) 2937–2941.
- [90] J.L. Antunes, P.W. Carmel, E.M. Housepian, M. Ferin, Luteinizing hormone-releasing hormone in human pituitary blood, *J. Neurosurg.* 49 (3) (1978) 382–386.
- [91] D.D. Braat, J. Schoemaker, Endocrinology of gonadotropin-releasing hormone induced cycles in hypothalamic amenorrhea: the role of the pulse dose, *Fertil. Steril.* 56 (6) (1991) 1054–1059.
- [92] M. Filicori, C. Flamigni, M.C. Meriggiola, P. Ferrari, L. Michelacci, E. Campaniello, A. Valdiserri, G. Cognigni, Endocrine response determines the clinical outcome of pulsatile gonadotropin-releasing hormone ovulation induction in different ovulatory disorders, *J. Clin. Endocrinol. Metab.* 72 (5) (1991) 965–972.

- [93] C.M. Blacker, Ovulation stimulation and induction, *Endocrinol. Metab. Clin. North Am.* 21 (1) (1992) 57–84.
- [94] T. Raivio, J. Falardeau, A. Dwyer, R. Quinton, F.J. Hayes, V.A. Hughes, L.W. Cole, S.H. Pearce, H. Lee, P. Boepple, W.F. Crowley, N. Pitteloud, Reversal of idiopathic hypogonadotropic hypogonadism, *N. Engl. J. Med.* 357 (9) (2007) 863–873.
- [95] M.D. Scheiber, J.H. Liu, The Use of Gonadotropin-Releasing Hormone to Induce Ovulation, in: F. Peter von Dadelszen (Ed), *Glob. libr. women's med*, 2011.
- [96] S.L. Jeffcoate, R.H. Greenwood, D.T. Holland, Blood and urine clearance of luteinizing hormone releasing hormone in man measured by radioimmunoassay, *J. Endocrinol.* 60 (2) (1974) 305–314.
- [97] S.C. Sealfon, H. Weinstein, R.P. Millar, Molecular mechanisms of ligand interaction with the gonadotropin-releasing hormone receptor, *Endocr. Rev.* 18 (2) (1997) 180–205.
- [98] J.B. Engel, A.V. Schally, Drug Insight: clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone, *Nat. Clin. Pract. Endocrinol. Metab.* 3 (2) (2007) 157–167.
- [99] N. Lahlou, J.C. Carel, J.L. Chaussain, M. Roger, Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics, *J. Pediatr. Endocrinol. Metab.* 13 (Suppl 1) (2000) 723–737.
- [100] S.E. Schagen, P.T. Cohen-Kettenis, H.A. Delemarre-van de Waal, S.E. Hannema, Efficacy and Safety of Gonadotropin-Releasing Hormone Agonist Treatment to Suppress Puberty in Gender Dysphoric Adolescents, *J. Sex. Med.* 13 (7) (2016) 1125–1132.
- [101] T. Brik, L.J.J.J. Vrouwenraets, M.C. de Vries, S.E. Hannema, Trajectories of Adolescents Treated with Gonadotropin-Releasing Hormone Analogues for Gender Dysphoria, *Arch. Sex. Behav.* 49 (7) (2020) 2611–2618.
- [102] G.G.F. Ramos, A.C.S. Mengai, C.A.T. Daltro, P.T. Cutrim, E. Zlotnik, A.P.A. Beck, Systematic Review: Puberty suppression with GnRH analogues in adolescents with gender incongruity, *J. Endocrinol. Invest.* 44 (6) (2021) 1151–1158.
- [103] U.B. Kaiser, P.M. Conn, W.W. Chin, Studies of gonadotropin-releasing hormone (GnRH) action using GnRH receptor-expressing pituitary cell lines, *Endocr. Rev.* 18 (1) (1997) 46–70.
- [104] H.M. Behre, J. Sandow, E. Nieschlag, Pharmacokinetics of the gonadotropin-releasing hormone agonist buserelin after injection of a slow-release preparation in normal men, *Arzneimittelforschung* 42 (1) (1992) 80–84.
- [105] T.K. Cheung, K.W. Lo, C.W. Lam, W. Lau, P.K. Lam, A crossover study of triptorelin and leuprolide acetate, *Fertil. Steril.* 74 (2) (2000) 299–305.
- [106] R.N. Clayton, L.C. Bailey, J. Cottam, D. Arkell, T.J. Perren, G.R. Blackledge, A radioimmunoassay for GnRH agonist analogue in serum of patients with prostate cancer treated with D-Ser (tBu)₆ AZA Gly₁₀ GnRH, *Clin. Endocrinol.* 22 (4) (1985) 453–462.
- [107] I.D. Cockshott, Clinical pharmacokinetics of goserelin, *Clin. Pharmacokinet.* 39 (1) (2000) 27–48.
- [108] A.M. Franssen, W.N. Willemsen, R.S. Corbey, W.H. Doesburg, A.J. van 't Veen, R. Rolland, Subcutaneous injection or infusion of gonadotropin releasing-hormone agonist buserelin in the treatment of enlarged uteri harboring leiomyomata, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 40 (3) (1991) 221–228.
- [109] M.R. Hoda, M.W. Kramer, A.S. Merseburger, M.V. Cronauer, Androgen deprivation therapy with Leuprolide acetate for treatment of advanced prostate cancer, *Expert Opin. Pharmacother.* 18 (1) (2017) 105–113.
- [110] J. Kong, F. Su, Y. Liu, Y. Yang, Y. Cao, J. Qiu, Y. Wang, L. Zhang, J. Wang, X. Cao, The pharmacokinetics of buserelin after intramuscular administration in pigs and cows, *BMC Vet. Res.* 18 (1) (2022) 136.
- [111] T. Lebre, M. Rouanne, O. Hublarov, V. Jinga, L. Petkova, R. Kotsev, I. Sinescu, P. Dutailly, Efficacy of triptorelin pamoate 11.25 mg administered subcutaneously for achieving medical castration levels of testosterone in patients with locally advanced or metastatic prostate cancer, *Ther. Adv. Urol.* 7 (3) (2015) 125–134.
- [112] J.L. Barron, R.P. Millar, D. Searle, Metabolic clearance and plasma half-disappearance time of D-TRP6 and exogenous luteinizing hormone-releasing hormone, *J. Clin. Endocrinol. Metab.* 54 (6) (1982) 1169–1173.
- [113] N. Heinrich, E. Albrecht, J. Sandow, U. Kertscher, D. Lorenz, J. Oehlke, H. Berger, Disposition of ³H-labelled buserelin continuously infused into rats, *Eur. J. Drug Metab. Pharmacokinet.* 21 (4) (1996) 345–350.
- [114] P. Periti, T. Mazzei, E. Mini, Clinical pharmacokinetics of depot leuprolide, *Clin. Pharmacokinet.* 41 (7) (2002) 485–504.
- [115] P. Whelan, Triptorelin embonate: a 6-month formulation for prostate cancer, *Expert Opin. Pharmacother.* 11 (17) (2010) 2929–2932.
- [116] M. Fu, X. Zhuang, T. Zhang, Y. Guan, Q. Meng, Y. Zhang, PEGylated leuprolide with improved pharmacokinetic properties, *Bioorg. Med. Chem.* 28 (4) (2020), 115306.
- [117] M.H. Perrin, J.E. Rivier, W.W. Vale, Radioligand assay for gonadotropin-releasing hormone: relative potencies of agonists and antagonists, *Endocrinology* 106 (4) (1980) 1289–1296.
- [118] D.H. Coy, F. Labrie, M. Savary, E.J. Coy, A.V. Schally, LH-releasing activity of potent LH-RH analogs in vitro, *Biochem. Biophys. Res. Commun.* 67 (2) (1975) 576–582.
- [119] F. Haviv, E.N. Bush, J. Knittle, J. Greer, LHRH antagonists, *Pharm. Biotechnol.* 11 (1998) 131–149.
- [120] C.F. Heyns, Triptorelin in the Treatment of Prostate Cancer, *American Journal of Cancer* 4 (2005) 169–183.
- [121] I. Nederpelt, V. Georgi, F. Schiele, K. Nowak-Reppel, A.E. Fernández-Montalván, A.P. IJzerman, L.H. Heitman, Characterization of 12 GnRH peptide agonists - a kinetic perspective, *Br. J. Pharmacol.* 173 (1) (2016) 128–141.
- [122] K. Maiti, J.H. Li, A.F. Wang, S. Acharjee, W.P. Kim, W.B. Im, H.B. Kwon, J. Y. Seong, GnRH-II analogs for selective activation and inhibition of non-mammalian and type-II mammalian GnRH receptors, *Mol. Cells* 16 (2) (2003) 173–179.
- [123] A.M. Padula, GnRH analogues-agonists and antagonists, *Anim. Reprod. Sci.* 88 (1–2) (2005) 115–126.
- [124] M.J. Karten, J.E. Rivier, Gonadotropin-releasing hormone analog design, Structure-function studies toward the development of agonists and antagonists: rationale and perspective, *Endocr Rev* 7 (1) (1986) 44–66.
- [125] A.V. Schally, Luteinizing hormone-releasing hormone analogs: their impact on the control of tumorigenesis, *Peptides* 20 (10) (1999) 1247–1262.
- [126] T. Cook, W.P. Sheridan, Development of GnRH antagonists for prostate cancer: new approaches to treatment, *Oncologist* 5 (2) (2000) 162–168.
- [127] A.V. Schally, LH-RH analogues: I, Their impact on reproductive medicine, *Gynecol Endocrinol* 13 (6) (1999) 401–409.
- [128] K.L. Herbst, Gonadotropin-releasing hormone antagonists, *Curr. Opin. Pharmacol.* 3 (6) (2003) 660–666.
- [129] G. Mezo, M. Manea, Luteinizing hormone-releasing hormone antagonists, *Expert Opin. Ther. Pat.* 19 (12) (2009) 1771–1785.
- [130] I. Huhtaniemi, R. White, C.A. McArdle, B.E. Persson, Will GnRH antagonists improve prostate cancer treatment? *Trends Endocrinol Metab* 20 (1) (2009) 43–50.
- [131] G. Emons, O. Ortmann, M. Becker, G. Irmer, B. Springer, R. Laun, F. Holzel, K. D. Schulz, A.V. Schally, High affinity binding and direct antiproliferative effects of LHRH analogues in human ovarian cancer cell lines, *Cancer Res.* 53 (22) (1993) 5439–5446.
- [132] B. Yu, J. Ruman, G. Christman, The role of peripheral gonadotropin-releasing hormone receptors in female reproduction, *Fertil. Steril.* 95 (2) (2011) 465–473.
- [133] S. Rama, A.J. Rao, Embryo implantation and GnRH antagonists: the search for the human placental GnRH receptor, *Hum. Reprod.* 16 (2) (2001) 201–205.
- [134] L.J. Duijkers, C. Klipping, W.N. Willemsen, D. Krone, E. Schneider, G. Niebch, R. Hermann, Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotrophin-releasing hormone antagonist Cetrorelix in healthy female volunteers, *Hum. Reprod.* 13 (9) (1998) 2392–2398.
- [135] L. Klotz, Pharmacokinetic and pharmacodynamic profile of degarelix for prostate cancer, *Expert Opin. Drug Metab. Toxicol.* 11 (11) (2015) 1795–1802.
- [136] J.J. Obery, B.M. Mannaerts, J.A. Huisman, C.J. Timmer, Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Antagon/Orgalutran). Part II. Dose-proportionality and gonadotropin suppression after multiple doses of ganirelix in healthy female volunteers, *Fertil. Steril.* 72 (6) (1999) 1006–1012.
- [137] S.L. Wong, D.T. Lau, S.A. Baughman, D. Menchaca, M.B. Garnick, Pharmacokinetics and pharmacodynamics of abarelix, a gonadotropin-releasing hormone antagonist, after subcutaneous continuous infusion in patients with prostate cancer, *Clin. Pharmacol. Ther.* 73 (4) (2003) 304–311.
- [138] J. Trachtenberg, M. Gittleman, C. Steidle, W. Barzell, W. Friedel, D. Pessis, N. Fotheringham, M. Campion, M.B. Garnick, Abarelix StudyA Group, A phase 3, multicenter, open label, randomized study of abarelix versus leuprolide plus daily antiandrogen in men with prostate cancer, *J. Urol.* 167 (4) (2002) 1670–1674.
- [139] K. Tomera, D. Gleason, M. Gittleman, W. Moseley, N. Zinner, M. Murdoch, M. Menon, M. Campion, M.B. Garnick, The gonadotropin-releasing hormone antagonist abarelix depot versus luteinizing hormone releasing hormone agonists leuprolide or goserelin: initial results of endocrinological and biochemical efficacies in patients with prostate cancer, *J. Urol.* 165 (5) (2001) 1585–1589.
- [140] M. Koch, C. Steidle, S. Brosman, A. Centeno, F. Gaylis, M. Campion, M.B. Garnick, Abarelix StudyA Group, An open-label study of abarelix in men with symptomatic prostate cancer at risk of treatment with LHRH agonists, *Urology* 62 (5) (2003) 877–882.
- [141] M. Gittleman, P.J. Pommerville, B.E. Persson, J.K. Jensen, T.K. Olesen, Degarelix StudyD Group, A 1-year, open label, randomized phase II dose finding study of degarelix for the treatment of prostate cancer in North America, *J. Urol.* 180 (5) (2008) 1986–1992.
- [142] L. Klotz, L. Boccon-Gibod, N.D. Shore, C. Andreou, B.E. Persson, P. Cantor, J. K. Jensen, T.K. Olesen, F.H. Schröder, The efficacy and safety of degarelix: a 12-month, comparative, randomized, open-label, parallel-group phase III study in patients with prostate cancer, *BJU Int.* 102 (11) (2008) 1531–1538.
- [143] S.F. Betz, Y.F. Zhu, C. Chen, R.S. Struthers, Non-peptide gonadotropin-releasing hormone receptor antagonists, *J. Med. Chem.* 51 (12) (2008) 3331–3348.
- [144] F.L. Tukun, D.E. Olberg, P.J. Riss, I. Haraldsen, A. Kaass, J. Klaveness, Recent Development of Non-Peptide GnRH Antagonists, *Molecules* 22 (12) (2017) 2188.
- [145] B. De, J.J. Plattner, E.N. Bush, H.S. Jae, G. Diaz, E.S. Johnson, T.J. Perun, LH-RH antagonists: design and synthesis of a novel series of peptidomimetics, *J. Med. Chem.* 32 (9) (1989) 2036–2038.
- [146] C. Chen, D. Wu, Z. Guo, Q. Xie, G.J. Reinhart, A. Madan, J. Wen, T. Chen, C. Q. Huang, M. Chen, Y. Chen, F.C. Tucci, M. Rowbottom, J. Pontillo, Y.F. Zhu, W. Wade, J. Saunders, H. Bozigian, R.S. Struthers, Discovery of sodium R-(+)-4-{2-[5-(2-fluoro-3-methoxyphenyl)-3-(2-fluoro-6-[trifluoromethyl]benzyl)-4-methyl-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-yl]-1-phenylethylamino}butyrate (elagolix), a potent and orally available nonpeptide antagonist of the human gonadotropin-releasing hormone receptor, *J. Med. Chem.* 51 (23) (2008) 7478–7485.
- [147] K. Miwa, T. Hitaka, T. Imada, S. Sasaki, M. Yoshimatsu, M. Kusaka, A. Tanaka, D. Nakata, S. Furuya, S. Endo, K. Hamamura, T. Kitazaki, Discovery of 1-{4-[1-(2,6-difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidin-6-yl]phenyl}-3-methoxyurea (TAK-385) as a potent, orally active, non-peptide antagonist of the human gonadotropin-releasing hormone receptor, *J. Med. Chem.* 54 (14) (2011) 4998–5012.

- [148] M. Tezuka, Y. Tamai, Y. Kuramochi, K. Kobayashi, N. Fushimi, S. Kiguchi, Pharmacological characterization of linzagolix, a novel, orally active, non-peptide antagonist of gonadotropin-releasing hormone receptors, *Clin. Exp. Pharmacol. Physiol.* 49 (10) (2022) 1082–1093.
- [149] A. Al-Hendy, L. Bradley, C.D. Owens, H. Wang, K.T. Barnhart, E. Feinberg, W. D. Schlaff, E.E. Puscheck, A. Wang, V. Gillispie, S. Hurtado, O. Muneyyirci-Delale, D.F. Archer, B.R. Carr, J.A. Simon, E.A. Stewart, Predictors of response for elagolix with add-back therapy in women with heavy menstrual bleeding associated with uterine fibroids, *Am. J. Obstet. Gynecol.* 224 (1) (2021) 72.e1–72.e50.
- [150] B.R. Carr, E.A. Stewart, D.F. Archer, A. Al-Hendy, L. Bradley, N.B. Watts, M. P. Diamond, J. Gao, C.D. Owens, K. Chwalisz, W.R. Duan, A.M. Soliman, M. B. Dufek, J.A. Simon, Elagolix Alone or With Add-Back Therapy in Women With Heavy Menstrual Bleeding and Uterine Leiomyomas: A Randomized Controlled Trial, *Obstet. Gynecol.* 132 (5) (2018) 1252–1264.
- [151] M. Ali, H.Y. Chen, Y.F. Chiang, O.A. Badary, S.M. Hsia, A. Al-Hendy, An evaluation of relugolix/estradiol/norethindrone acetate for the treatment of heavy menstrual bleeding associated with uterine fibroids in premenopausal women, *Expert Opin. Pharmacother.* 23 (4) (2022) 421–429.
- [152] J. Donnez, H.S. Taylor, E.A. Stewart, L. Bradley, E. Marsh, D. Archer, A. Al-Hendy, F. Petraglia, N. Watts, J.P. Gotteland, E. Bestel, P. Terrill, E. Loumaye, A. Humberstone, E. Garner, Linzagolix with and without hormonal add-back therapy for the treatment of symptomatic uterine fibroids: two randomised, placebo-controlled, phase 3 trials, *Lancet* 400 (10356) (2022) 896–907.
- [153] O. Pohl, L. Marchand, D. Bell, J.P. Gotteland, Effects of combined GnRH receptor antagonist linzagolix and hormonal add-back therapy on vaginal bleeding-delayed add-back onset does not improve bleeding pattern, *Reprod. Sci.* 27 (4) (2020) 988–995.
- [154] R.S. Struthers, Q. Xie, S.K. Sullivan, G.J. Reinhart, T.A. Kohout, Y.F. Zhu, C. Chen, X.J. Liu, N. Ling, W. Yang, R.A. Maki, A.K. Bonneville, T.K. Chen, H.P. Bozigian, Pharmacological characterization of a novel nonpeptide antagonist of the human gonadotropin-releasing hormone receptor, NBI-42902, *Endocrinology* 148 (2) (2007) 857–867.
- [155] O. Panknin, A. Wagenfeld, W. Bone, E. Bender, K. Nowak-Reppel, A.E. Fernández-Montalván, R. Nubbemeyer, S. Bäurle, S. Ring, N. Schmees, O. Prien, M. Schäfer, C. Friedrich, T.M. Zollner, A. Steinmeyer, T. Mueller, G. Langer, Discovery and Characterization of BAY 1214784, an Orally Available Spiroindoline Derivative Acting as a Potent and Selective Antagonist of the Human Gonadotropin-Releasing Hormone Receptor as Proven in a First-In-Human Study in Postmenopausal Women, *J. Med. Chem.* 63 (20) (2020) 11854–11881.
- [156] S. Li, S. Ke, C. Yang, J. Chen, Y. Xiong, L. Zheng, H. Liu, L. Hong, A Ligand-and-structure Dual-driven Deep Learning Method for the Discovery of Highly Potent GnRH1R Antagonist to treat Uterine Diseases, *arXiv:2207.11547* (2022).
- [157] W. Yan, L. Cheng, W. Wang, C. Wu, X. Yang, X. Du, L. Ma, S. Qi, Y. Wei, Z. Lu, S. Yang, Z. Shao, Structure of the human gonadotropin-releasing hormone receptor GnRH1R reveals an unusual ligand binding mode, *Nat. Commun.* 11 (1) (2020) 5287.
- [158] J. Cui, R.G. Smith, G.R. Mount, J.L. Lo, J. Yu, T.F. Walsh, S.B. Singh, R.J. DeVita, M.T. Goulet, J.M. Schaeffer, K. Cheng, Identification of Phe313 of the gonadotropin-releasing hormone (GnRH) receptor as a site critical for the binding of nonpeptide GnRH antagonists, *Mol. Endocrinol.* 14 (5) (2000) 671–681.
- [159] G.J. Reinhart, Q. Xie, X.J. Liu, Y.F. Zhu, J. Fan, C. Chen, R.S. Struthers, Species selectivity of nonpeptide antagonists of the gonadotropin-releasing hormone receptor is determined by residues in extracellular loops II and III and the amino terminus, *J. Biol. Chem.* 279 (33) (2004) 34115–34122.
- [160] S.F. Betz, G.J. Reinhart, F.M. Lio, C. Chen, R.S. Struthers, Overlapping, nonidentical binding sites of different classes of nonpeptide antagonists for the human gonadotropin-releasing hormone receptor, *J. Med. Chem.* 49 (2) (2006) 637–647.
- [161] G.J. Sullivan, Y. Bai, J. Fletcher, I. Wilmut, Induced pluripotent stem cells: epigenetic memories and practical implications, *Mol. Hum. Reprod.* 16 (12) (2010) 880–885.
- [162] X. Yang, G. Lin, A. Xia, J. Liu, S. Zhang, P. Zhou, Y. Wang, J. Zhang, Y. Zhou, P. Chen, T. Zheng, L. Li, S. Yang, Discovery of Small Molecule Agonist of Gonadotropin-Releasing Hormone Receptor (GnRH1R), *J. Chem. Inf. Model.* 62 (20) (2022) 5009–5022.
- [163] M. Shebley, A.R. Polepally, A. Nader, J.W. Ng, I. Winzenborg, C.E. Klein, P. Noertersheuser, M.A. Gibbs, N.M. Mostafa, Clinical Pharmacology of Elagolix: An Oral Gonadotropin-Releasing Hormone Receptor Antagonist for Endometriosis, *Clin. Pharmacokinet.* 59 (3) (2020) 297–309.
- [164] D.B. MacLean, H. Shi, H.M. Faessel, F. Saad, Medical Castration Using the Investigational Oral GnRH Antagonist TAK-385 (Relugolix): Phase 1 Study in Healthy Males, *J. Clin. Endocrinol. Metab.* 100 (12) (2015) 4579–4587.
- [165] S.J. Keam, Linzagolix: First Approval, *Drugs* 82 (12) (2022) 1317–1325.
- [166] O. Pohl, K. Baron, M. Riggs, J. French, R. Garcia, J.P. Gotteland, A model-based analysis to guide gonadotropin-releasing hormone receptor antagonist use for management of endometriosis, *Br. J. Clin. Pharmacol.* 88 (5) (2022) 2359–2371.
- [167] O. Pohl, L. Marchand, N. Fawkes, J.P. Gotteland, E. Loumaye, Gonadotropin-Releasing Hormone Receptor Antagonist Mono- and Combination Therapy With Estradiol/Norethindrone Acetate Add-Back: Pharmacodynamics and Safety of OBE2109, *J. Clin. Endocrinol. Metab.* 103 (2) (2018) 497–504.
- [168] Y.N. Lamb, Elagolix: First Global Approval, *Drugs* 78 (14) (2018) 1501–1508.
- [169] J. Donnez, H.S. Taylor, R.N. Taylor, M.D. Akin, T.F. Tatarchuk, K. Wilk, J. P. Gotteland, V. Lecomte, E. Bestel, Treatment of endometriosis-associated pain with linzagolix, an oral gonadotropin-releasing hormone-antagonist: a randomized clinical trial, *Fertil. Steril.* 114 (1) (2020) 44–55.
- [170] A. Markham, Relugolix: First Global Approval, *Drugs* 79 (6) (2019) 675–679.
- [171] A. Al-Hendy, A.S. Lukes, A.N. Poindexter, R. Venturella, C. Villarroel, H.O. D. Critchley, Y. Li, L. McKain, J.C. Arjona Ferreira, A.G.M. Langenberg, R. B. Wagman, E.A. Stewart, Treatment of Uterine Fibroid Symptoms with Relugolix Combination Therapy, *N. Engl. J. Med.* 384 (7) (2021) 630–642.
- [172] Y. Osuga, Y. Seki, M. Tanimoto, T. Kusumoto, K. Kudou, N. Terakawa, Relugolix, an oral gonadotropin-releasing hormone receptor antagonist, reduces endometriosis-associated pain in a dose-response manner: a randomized, double-blind, placebo-controlled study, *Fertil. Steril.* 115 (2) (2021) 397–405.
- [173] R.L. Reid, G.R. Leopold, S.S. Yen, Induction of ovulation and pregnancy with pulsatile luteinizing hormone releasing factor: dosage and mode of delivery, *Fertil. Steril.* 36 (5) (1981) 553–559.
- [174] J.B. Martin, Molecular genetics in neurology, *Ann. Neurol.* 34 (6) (1993) 757–773.
- [175] P. Humaidan, H.E. Bredkjaer, L. Bungum, M. Bungum, M.L. Grøndahl, L. Westergaard, C.Y. Andersen, GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study, *Hum. Reprod.* 20 (5) (2005) 1213–1220.
- [176] R.F. Casper, Introduction: Gonadotropin-releasing hormone agonist triggering of final follicular maturation for in vitro fertilization, *Fertil. Steril.* 103 (4) (2015) 865–866.
- [177] M.A. Mittelman-Smith, A.M. Wong, A.S. Kathiresan, P.E. Micevych, Classical and membrane-initiated estrogen signaling in an in vitro model of anterior hypothalamic kisspeptin neurons, *Endocrinology* 156 (6) (2015) 2162–2173.
- [178] F. Olivennes, J.S. Cunha-Filho, R. Fanchin, P. Bouchard, R. Frydman, The use of GnRH antagonists in ovarian stimulation, *Hum. Reprod. Update* 8 (3) (2002) 279–290.
- [179] P. Li, Y. Li, C.L. Yang, Gonadotropin releasing hormone agonist treatment to increase final stature in children with precocious puberty: a meta-analysis, *Medicine (Baltimore)* 93 (27) (2014) e260.
- [180] P. Thornton, L.A. Silverman, M.E. Geffner, E.K. Neely, E. Gould, T.M. Danoff, Review of outcomes after cessation of gonadotropin-releasing hormone agonist treatment of girls with precocious puberty, *Pediatr. Endocrinol. Rev.* 11 (3) (2014) 306–317.
- [181] R.P. Millar, C.L. Newton, Current and future applications of GnRH, kisspeptin and neurokinin B analogues, *Nat. Rev. Endocrinol.* 9 (8) (2013) 451–466.
- [182] L. Platteeuw, T. D'Hooghe, Novel agents for the medical treatment of endometriosis, *Curr. Opin. Obstet. Gynecol.* 26 (4) (2014) 243–252.
- [183] G. Zito, S. Luppi, E. Giolo, M. Martinelli, I. Venturin, G. Di Lorenzo, G. Ricci, Medical treatments for endometriosis-associated pelvic pain, *Biomed Res. Int.* 2014 (2014), 191967.
- [184] P. Limonta, M.M. Marelli, S. Mai, M. Motta, L. Martini, R.M. Moretti, GnRH Receptors in Cancer: From Cell Biology to Novel Targeted Therapeutic Strategies, *Endocr. Rev.* 33 (5) (2012) 784–811.
- [185] K.N. Khan, M. Kitajima, K. Hiraki, A. Fujishita, M. Nakashima, T. Ishimaru, H. Masuzaki, Cell proliferation effect of GnRH agonist on pathological lesions of women with endometriosis, adenomyosis and uterine myoma, *Hum. Reprod.* 25 (11) (2010) 2878–2890.
- [186] K. Nirgianakis, N.A. Bersinger, B. McKinnon, P. Kostov, S. Imboden, M.D. Mueller, Regression of the inflammatory microenvironment of the peritoneal cavity in women with endometriosis by GnRHa treatment, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 170 (2) (2013) 550–554.
- [187] C. Gründker, G. Emons, The Role of Gonadotropin-Releasing Hormone in Cancer Cell Proliferation and Metastasis, *Front Endocrinol (Lausanne)* 8 (2017) 187.
- [188] SGO Clinical Practice Endometrial Cancer Working Group, W.M. Burke, J. Orr, M. Leita, E. Salom, P. Gehrig, A.B. Olawaiye, M. Brewer, D. Boruta, J. Villella, T. Herzog, F. Abu Shahin, Society of Gynecologic Oncology Clinical Practice Committee, Endometrial cancer: a review and current management strategies: part I, *Gynecol Oncol* 134(2) (2014) 385–92.
- [189] L.A. Torre, B. Trabert, C.E. DeSantis, K.D. Miller, G. Samimi, C.D. Runowicz, M. M. Gaudet, A. Jemal, R.L. Siegel, Ovarian cancer statistics, 2018, *CA Cancer J. Clin.* 68 (4) (2018) 284–296.
- [190] E. Chatzaki, C.M.R. Bax, K.A. Eidne, L. Anderson, J.G. Grudzinskas, C. J. Gallagher, The expression of gonadotropin-releasing hormone and its receptor in endometrial cancer, and its relevance as an autocrine growth factor, *Cancer Res.* 56 (9) (1996) 2059–2065.
- [191] S. Shibata, H. Sato, H. Ota, A. Karube, O. Takahashi, T. Tanaka, Involvement of annexin V in antiproliferative effects of gonadotropin-releasing hormone agonists on human endometrial cancer cell line, *Gynecol. Oncol.* 66 (2) (1997) 217–221.
- [192] N. Eicke, A.R. Gunthert, G. Emons, C. Grundker, GnRH-II agonist [D-Lys6]GnRH-II inhibits the EGF-induced mitogenic signal transduction in human endometrial and ovarian cancer cells, *Int. J. Oncol.* 29 (5) (2006) 1223–1229.
- [193] S. Fister, A.R. Gunthert, B. Aicher, K.W. Paulini, G. Emons, C. Grundker, GnRH-II antagonists induce apoptosis in human endometrial, ovarian, and breast cancer cells via activation of stress-induced MAPKs p38 and JNK and proapoptotic protein Bax, *Cancer Res.* 69 (16) (2009) 6473–6481.
- [194] P. Rawla, Epidemiology of Prostate Cancer, *World J Oncol* 10 (2) (2019) 63–89.
- [195] F. Fontana, M. Marzagalli, M. Montagnani Marelli, M. Raimondi, R.M. Moretti, P. Limonta, Gonadotropin-Releasing Hormone Receptors in Prostate Cancer: Molecular Aspects and Biological Functions, *Int. J. Mol. Sci.* 21 (24) (2020) 9511.
- [196] F. Zengerling, J.J. Jakob, S. Schmidt, J.J. Meerpohl, A. Blümle, C. Schmucker, B. Mayer, F. Kunath, Degarelix for treating advanced hormone-sensitive prostate cancer, *Cochrane Database Syst. Rev.* 8 (8) (2021) CD012548.
- [197] F. Saad, N.D. Shore, Relugolix: a novel androgen deprivation therapy for management of patients with advanced prostate cancer, *Ther Adv Med Oncol* 13 (2021), 1758835921998586.

- [198] M. Huerta-Reyes, G. Maya-Núñez, M.A. Pérez-Solis, E. López-Muñoz, N. Guillén, J.C. Olivo-Marin, A. Aguilar-Rojas, Treatment of Breast Cancer With Gonadotropin-Releasing Hormone Analogs, *Front. Oncol.* 9 (2019) 943.
- [199] S. Park, J.M. Han, J. Cheon, J.I. Hwang, J.Y. Seong, Apoptotic death of prostate cancer cells by a gonadotropin-releasing hormone-II antagonist, *PLoS One* 9 (6) (2014) e99723.
- [200] L. Suo, X. Chang, N. Xu, H. Ji, The Anti-proliferative Activity of GnRH Through Downregulation of the Akt/ERK Pathways in Pancreatic Cancer, *Front Endocrinol (Lausanne)* 10 (2019) 370.
- [201] M. Montagnani Marelli, R.M. Moretti, S. Mai, O. Müller, J.C. Van Groeninghen, P. Limonta, Novel insights into GnRH receptor activity: role in the control of human glioblastoma cell proliferation, *Oncol. Rep.* 21 (5) (2009) 1277–1282.
- [202] J. Engel, G. Emons, J. Pinski, A.V. Schally, AEZS-108: a targeted cytotoxic analog of LHRH for the treatment of cancers positive for LHRH receptors, *Expert Opin. Invest. Drugs* 21 (6) (2012) 891–899.
- [203] C. Gründker, G. Emons, Role of Gonadotropin-Releasing Hormone (GnRH) in Ovarian Cancer, *Cells* 10 (2) (2021) 437.
- [204] G. Emons, C. Gründker, The Role of Gonadotropin-Releasing Hormone (GnRH) in Endometrial Cancer, *Cells* 10 (2) (2021) 292.
- [205] P. Limonta, M. Manea, Gonadotropin-releasing hormone receptors as molecular therapeutic targets in prostate cancer: Current options and emerging strategies, *Cancer Treat. Rev.* 39 (6) (2013) 647–663.
- [206] H. Roness, Z. Gavish, Y. Cohen, D. Meirou, Ovarian follicle burnout: a universal phenomenon? *Cell Cycle* 12 (20) (2013) 3245–3246.
- [207] Z. Blumenfeld, How to preserve fertility in young women exposed to chemotherapy? The role of GnRH agonist cotreatment in addition to cryopreservation of embryos, oocytes, or ovaries, *Oncologist* 12 (9) (2007) 1044–1054.
- [208] N. Mahajan, Fertility preservation in female cancer patients: An overview, *J Hum Reprod Sci* 8 (1) (2015) 3–13.
- [209] E.R. Cardozo, A.P. Thomson, A.E. Karmon, K.A. Dickinson, D.L. Wright, M. E. Sabatini, Ovarian stimulation and in-vitro fertilization outcomes of cancer patients undergoing fertility preservation compared to age matched controls: a 17-year experience, *J. Assist. Reprod. Genet.* 32 (4) (2015) 587–596.
- [210] Z. Blumenfeld, A. Evron, Preserving fertility when choosing chemotherapy regimens - the role of gonadotropin-releasing hormone agonists, *Expert Opin. Pharmacother.* 16 (7) (2015) 1009–1020.
- [211] H. Chen, L. Xiao, J. Li, L. Cui, W. Huang, Adjuvant gonadotropin-releasing hormone analogues for the prevention of chemotherapy-induced premature ovarian failure in premenopausal women, *Cochrane Database Syst. Rev.* 3 (3) (2019) CD008018.
- [212] V.L. Burnham, J.E. Thornton, Luteinizing hormone as a key player in the cognitive decline of Alzheimer's disease, *Horm. Behav.* 76 (2015) 48–56.
- [213] K. Andersen, L.J. Launer, M.E. Dewey, L. Letenneur, A. Ott, J.R. Copeland, J. F. Dartigues, P. Kragh-Sorensen, M. Baldereschi, C. Brayne, A. Lobo, J. M. Martinez-Lage, T. Stijnen, A. Hofman, Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies, *EURODEM Incidence Research Group, Neurology* 53 (9) (1999) 1992–1997.
- [214] M.A. Arevalo, I. Azcoitia, L.M. Garcia-Segura, The neuroprotective actions of oestradiol and oestrogen receptors, *Nat. Rev. Neurosci.* 16 (1) (2015) 17–29.
- [215] J. Prange-Kiel, H. Jarry, M. Schoen, P. Kohlmann, C. Lohse, L. Zhou, G.M. Rune, Gonadotropin-releasing hormone regulates spine density via its regulatory role in hippocampal estrogen synthesis, *J. Cell Biol.* 180 (2) (2008) 417–426.
- [216] K.J. Bryan, J.C. Mudd, S.L. Richardson, J. Chang, H.G. Lee, X. Zhu, M.A. Smith, G. Casadesus, Down-regulation of serum gonadotropins is as effective as estrogen replacement at improving menopause-associated cognitive deficits, *J. Neurochem.* 112 (4) (2010) 870–881.
- [217] A. Hashizume, M. Katsuno, K. Suzuki, A. Hirakawa, Y. Hijikata, S. Yamada, T. Inagaki, H. Banno, G. Sobue, Long-term treatment with leuporelin for spinal and bulbar muscular atrophy: natural history-controlled study, *J. Neurol. Neurosurg. Psychiatry* 88 (12) (2017) 1026–1032.
- [218] W.R. Kennedy, M. Alter, J.H. Sung, Progressive proximal spinal and bulbar muscular atrophy of late onset, A sex-linked recessive trait, *Neurology* 18 (7) (1968) 671–680.
- [219] A. Hashizume, M. Katsuno, K. Suzuki, H. Banno, Y. Takeuchi, M. Kawashima, N. Suga, T. Mano, A. Araki, Y. Hijikata, A. Hirakawa, G. Sobue, J.s. group, Efficacy and safety of leuporelin acetate for subjects with spinal and bulbar muscular atrophy: pooled analyses of two randomized-controlled trials, *J. Neurol.* 266 (5) (2019) 1211–1221.
- [220] C. Díaz Galindo, B. Gómez-González, E. Salinas, D. Calderón-Vallejo, I. Hernández-Jasso, E. Bautista, J.L. Quintanar, Leuprolide acetate induces structural and functional recovery of injured spinal cord in rats, *Neural Regen. Res.* 10 (11) (2015) 1819–1824.
- [221] M. Altamira-Camacho, D. Medina-Aguinaga, Y. Cruz, D. Calderón-Vallejo, K. Kovacs, F. Rotondo, J.L. Quintanar, Leuprolide Acetate, a GnRH Agonist, Improves the Neurogenic Bowel in Ovariectomized Rats with Spinal Cord Injury, *Dig. Dis. Sci.* 65 (2) (2020) 423–430.
- [222] J.L. Quintanar, C. Díaz-Galindo, D. Calderón-Vallejo, I. Hernández-Jasso, F. Rojas, D. Medina-Aguinaga, C. Olvera-Sandoval, Neurological improvement in patients with chronic spinal cord injury treated with leuprolide acetate, an agonist of GnRH, *Acta Neurobiol. Exp. (Wars)* 78 (4) (2018) 352–357.
- [223] I. Guzmán-Soto, E. Salinas, I. Hernández-Jasso, J.L. Quintanar, Leuprolide acetate, a GnRH agonist, improves experimental autoimmune encephalomyelitis: a possible therapy for multiple sclerosis, *Neurochem. Res.* 37 (10) (2012) 2190–2197.
- [224] K.M. Krysko, J.S. Graves, R. Dobson, A. Altintas, M.P. Amato, J. Bernard, S. Bonavita, R. Bove, P. Cavalla, M. Clerico, T. Corona, A. Doshi, Y. Fragoso, D. Jacobs, V. Jokubaitis, D. Landi, G. Llamasa, E.E. Longbrake, E. Maillart, M. Marta, L. Midaglia, S. Shah, M. Tintore, A. van der Walt, R. Voskuhl, Y. Wang, R.K. Zabad, B. Zeydan, M. Houtchens, K. Hellwig, Sex effects across the lifespan in women with multiple sclerosis, *Ther. Adv. Neurol. Disord.* 13 (2020), 1756286420936166.
- [225] K. Sakurai, K. Shinohara, T. Imai, Y. Yamano, Y. Hasegawa, Severe Multiple Sclerosis Manifesting upon GnRH Agonist Therapy for Uterine Fibroids, *Intern. Med.* 59 (23) (2020) 3093–3096.
- [226] A. Villa, E. Vegeto, A. Poletti, A. Maggi, Estrogens, Neuroinflammation, and Neurodegeneration, *Endocr. Rev.* 37 (4) (2016) 372–402.
- [227] G. Varytė, J. Zakarevičienė, D. Ramašauskaitė, D. Laužikienė, A. Arlauskienė, Pregnancy and Multiple Sclerosis: An Update on the Disease Modifying Treatment Strategy and a Review of Pregnancy's Impact on Disease Activity, *Medicina (Kaunas)* 56 (2) (2020) 49.