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The Low-Molecular-Weight Ligands of the Gonadotropin Receptors as the New Generation of the Regulators of the Reproductive Functions and Steroidogenesis

*Alexander O. Shpakov, Kira V. Derkach,
Andrey A. Bakhtyukov and Dmitry V. Dar'in*

Abstract

In clinic, the luteinizing (LH) and follicle-stimulating (FSH) hormones and human chorionic gonadotropin (hCG) are used to treat reproductive dysfunctions and in assisted reproductive technology. They are the $\alpha\beta$ -heterodimeric complexes and specifically bind to ectodomain of G protein-coupled LH and FSH receptors. This leads to activation of many signaling cascades; some of which are responsible for steroidogenesis, folliculogenesis, and spermatogenesis, while the others, such as β -arrestin pathways, trigger the downregulation of gonadotropin receptors. A low selectivity of the intracellular signaling of gonadotropins and a large number of their isoforms are the main causes of undesirable effects of gonadotropins, limiting their clinical applications. Unlike gonadotropins, the low-molecular-weight (LMW) ligands interact with an allosteric site located in the transmembrane domain of the LH and FSH receptors and selectively activate the certain signaling pathway, preventing a number of side effects of gonadotropins. The LMW ligands are characterized by activity of the full and inverse agonists and neutral antagonists, as well as the positive and negative modulators, and they have the *in vivo* activity, including when administered orally. This review focuses on the advances in the development of LMW allosteric ligands of the LH and FSH receptors and the prospects for their use in reproductive medicine.

Keywords: sex steroid hormone, steroidogenesis, low-molecular-weight agonist, luteinizing hormone, follicle-stimulating hormone, receptor of luteinizing hormone

1. Introduction

The most important areas of clinical applications of the gonadotropins, such as the luteinizing (LH) and follicle-stimulating (FSH) hormones and human chorionic gonadotropin (hCG), are (i) the stimulation of the steroidogenesis,

folliculogenesis, and spermatogenesis in patients with the dysfunctions in the hypothalamo-pituitary-gonadal axis, (ii) the induction of ovulation in the assisted reproductive technologies, and (iii) the treatment of sex hormone-dependent tumors [1–4]. The gonadotropins with LH activity are isolated from the urine of pregnant women (the urinary forms of hCG) or produced in the specialized cellular cultures (the recombinant forms of LH and hCG), while FSH is isolated from the urine of postmenopausal women (the urinary forms of FSH) or produced by genetic engineering approaches (the recombinant forms of FSH) [1, 5, 6]. Despite the fact that these forms of gonadotropins are widely used in the clinic, they have the significant side effects. In the case of urinary forms of hCG and FSH, the main disadvantages are the presence of biologically active impurities in the gonadotropin preparations and a low degree of its standardization [2, 7]. The placental hCG differs significantly in both the structure and functions from the LH and sulfated hCG which are secreted by the pituitary gonadotrophs and circulate in the blood of adult men and women [2, 8]. Furthermore, the placental hCG is produced only during pregnancy and regulates the growth and development of the embryo [9]. The urinary FSH contains mainly highly glycosylated forms of this gonadotropin with the reduced activity, which associated with the impaired reproductive functions and infertility at the postmenopausal period [10]. At the same time, the recombinant forms of gonadotropins differ from their natural forms in the posttranslational modifications, primarily in the number, structure, and charge of N-glycans, which significantly changes their specific biological activity and pharmacological profile. All this not only significantly limits the use of natural and recombinant forms of gonadotropins in the treatment of androgen deficiency, hypogonadotropic hypogonadism, and amenorrhea but also reduces their effectiveness in the controlled induction of ovulation and in other assisted reproductive technologies.

Thus, one of the urgent tasks of reproductive medicine is to minimize the side effects of gonadotropins and to increase their effectiveness and specificity. An alternative approach is the development of a new generation of the selective regulators of the LH and FSH receptors, the most suitable among which are the low-molecular-weight (LMW) allosteric ligands of these receptors.

2. The LH and FSH receptors and their binding with the gonadotropins and the low-molecular-weight allosteric ligands

The LH and FSH receptors belong to the δ group of the rhodopsin family of the G protein-coupled receptors (GPCR) and contain seven membrane-penetrating hydrophobic regions that form the heptahelical transmembrane channel [11–16]. Unlike most GPCRs with a short extracellular N-terminal region, the gonadotropin receptors have a large-size extracellular domain (an ectodomain) containing the leucine-rich repeats (LRRs). The LRRs are involved in the formation of an orthosteric site responsible for a high-affinity binding of the receptors with gonadotropins. The gonadotropins are the $\alpha\beta$ -heterodimeric complexes, in which the α -subunit encoded by a single gene is identical in all gonadotropins, while the β -subunits are encoded by separate genes and differ in the primary structure and modifications. The $\alpha\beta$ -heterodimeric complexes of gonadotropins are stabilized by the cystine knots that ensure a close contact between the central regions of the α - and β -subunits [12, 16]. The β -subunit is responsible for the specificity of gonadotropins binding with the LH and FSH receptors, while the α -subunit provides the physical contacts between the ligand-bound ectodomain and the transmembrane domain. The binding of gonadotropin with an ectodomain induces conformational

changes in both the transmembrane channel and the intracellular regions of the LH and FSH receptors, which are responsible for their functional coupling with the heterotrimeric G proteins (G_s , $G_{q/11}$, and $G_{i/o}$) and β -arrestins, resulting in the activation of a large number of the intracellular signaling cascades and the transcriptional factors [15–18] (**Figure 1**).

By activating the G_s proteins, the gonadotropins stimulate the enzyme adenylyl cyclase (AC), which leads to an increase in the intracellular cAMP levels and the activation of protein kinase A (PKA) and exchange protein directly activated by cyclic AMP (Epac). The stimulation of PKA leads to activation of the transcriptional factor cAMP-response element binding protein (CREB), which controls the expression of a large number of PKA-dependent genes, while the activation of Epac induces the stimulation of phosphatidylinositol-3-kinase, mitogen-activated protein kinases (MAPKs), and the other effector enzymes. The gonadotropin-induced activation of the cAMP-dependent pathways is the key mechanism for triggering the steroidogenesis, spermatogenesis, and folliculogenesis (**Figure 1**). By activating $G_{q/11}$ proteins, the gonadotropins stimulate the phosphoinositide-specific phospholipase C β (PLC β), which catalyzes the formation of inositol-3,4,5-triphosphate and diacylglycerol, the important second messengers. This induces the activation of calcium-dependent signaling and different isoforms of protein kinase C [15–19]. A specific interaction between the β -arrestins and the GPCR kinases-phosphorylated sites located within the intracellular loops of the LH and FSH receptors induces G

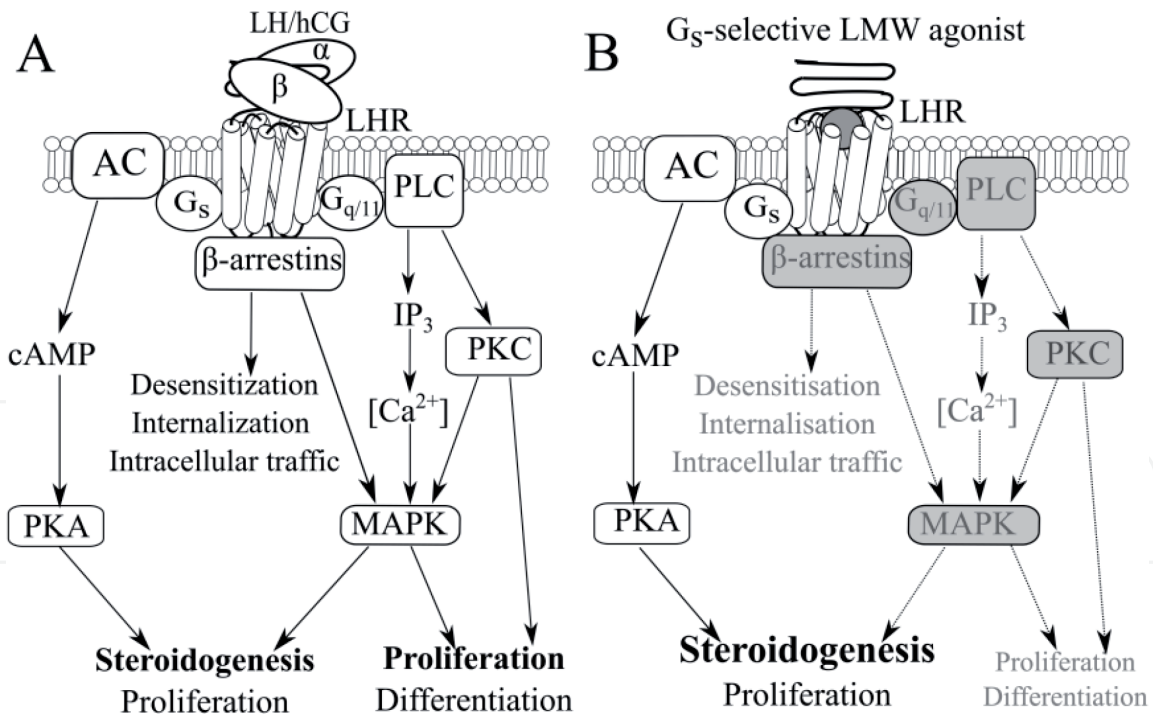


Figure 1.
The signaling pathways of the gonadotropins with LH-like activity and the G_s protein-selective LMW agonists. (A) The gonadotropins, LH and hCG, specifically bind to an ectodomain of LH receptor, which triggers the conformational changes in its serpentine domain and provokes the interaction of the intracellular regions of the receptor with the G proteins (G_s and $G_{q/11}$) and β -arrestins. The result of this is activation of the cAMP-dependent and phosphoinositide signaling pathways and the cascade of mitogen-activated protein kinases, which control the steroidogenesis, growth, differentiation, and apoptosis in the cells of reproductive tissues. A specific interaction of gonadotropin-activated LH receptor with β -arrestins induces the endocytosis of LH receptor; its degradation, or recycling. (B) The G_s -selective LMW agonists bind to the allosteric site located within the transmembrane channel of LH receptor and activate the G_s protein, triggering cAMP-signaling pathways. At the same time, these agonists do not have a significant effect on other signaling pathways. Abbreviations: AC, adenylyl cyclase; hCG, human chorionic gonadotropin; LH, luteinizing hormone; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PLC, phosphoinositide-specific phospholipase C β .

protein-independent stimulation of the MAPK cascade and is involved in the internalization, endocytosis, and recyclization of the gonadotropin receptor complexes [18–22]. The Ca^{2+} - and β -arrestin-dependent pathways, as well as the AC signaling system, are involved in the regulation of the synthesis and secretion of sex steroid hormones and also control the growth, differentiation, and survival of the testicular and ovarian cells (**Figure 1**). About two-thirds of gonadotropin-dependent genes are regulated through cAMP-dependent mechanisms, while the expression of another third of the genes is regulated through cAMP-independent mechanisms [23].

The choice of the intracellular signaling pathway depends on the stability and the ratio of active conformations of the LH and FSH receptors, which, in turn, is determined by (i) the type of gonadotropin and the structural features of its α - and β -subunits (the N-glycosylation, site-specific proteolysis, etc.), (ii) the structural features of the LH and FSH receptors (the posttranslational modifications, mutations, polymorphisms, etc.), and their ability to form the dimeric and oligomeric complexes, as well as (iii) the functional activity of the downstream regulatory and accessory proteins (the G proteins, β -arrestins, etc.) [6, 19, 24, 25]. A specific binding of gonadotropin to ectodomain generates a large set of the active conformations of receptor, which triggers some intracellular pathways at once and, as a result, induces multiple cell responses. For example, the placental hCG with a high efficiency stimulates the cAMP-dependent signaling pathways, being a powerful activator of the steroidogenesis, but its stimulating effect on the $\text{G}_{q/11}$ -mediated signaling is realized to a much lesser extent. Moreover, the recombinant LH with a high efficiency stimulates the PLC β - and β -arrestin-dependent signaling pathways, but weakly, in comparison with hCG, stimulates the cAMP-signaling and steroidogenesis [18].

Unlike gonadotropins, the ligands of the allosteric site that is located within the transmembrane channel of the LH and FSH receptors more selectively regulate the intracellular signaling cascades. This is due to the fact that, by binding to the allosteric site, they stabilize, as a rule, only one conformation of the gonadotropin receptor, either active or inactive. In the first case, they function as the full allosteric agonists or as the positive allosteric modulators (PAMs), enhancing the stimulating effects of gonadotropins, or as the ago-PAM, combining the effects of allosteric agonists and “pure” PAMs. In the second case, they function as the allosteric antagonists or as the negative allosteric modulators (NAMs) that prevent the activation of the LH and FSH receptors by gonadotropins [26]. Since the binding sites for the gonadotropins and the LMW allosteric ligands do not overlap, there is no competition between them. Due to this, in the case of co-administration of the gonadotropin and LMW agonist, their stimulating effects can be additive and even synergistic. Below we consider the most effective LMW allosteric ligands of the LH and FSH receptors, which were developed by us and the other authors.

3. Thienopyrimidine-based low-molecular-weight agonists of the luteinizing hormone receptor

Screening of a large number of organic compounds allowed to identify the 1,3,5-substituted pyrazole and terphenyl derivatives, which have the activity of the full and inverse allosteric agonists of LH receptor [27–30]. It was shown that a derivative of terphenyl, the compound LUF5771, inhibited the gonadotropin- and LMW agonist-induced stimulation of LH receptor, indicating that

the LUF5771 belongs to the inverse agonists. This compound can be used as a prototype to develop the contraceptives and the anticancer drugs for treatment of hormone-dependent tumors [29]. The 1,3,5-substituted pyrazole derivative, 8-(1-(4-(*tert*-butyl)phenyl)-3-(pyridin-3-yl)-1*H*-pyrazol-5-yl)-2-(4-hydroxybenzyl)-4-oxooctanamide (1) (**Figure 2**), had activity of the full agonist of LH receptor [27]. It stimulated the AC activity (EC_{50} , 20 nM) and increased the synthesis and secretion of testosterone by the Leydig cells (ED_{50} , 1.31 μ M), and when

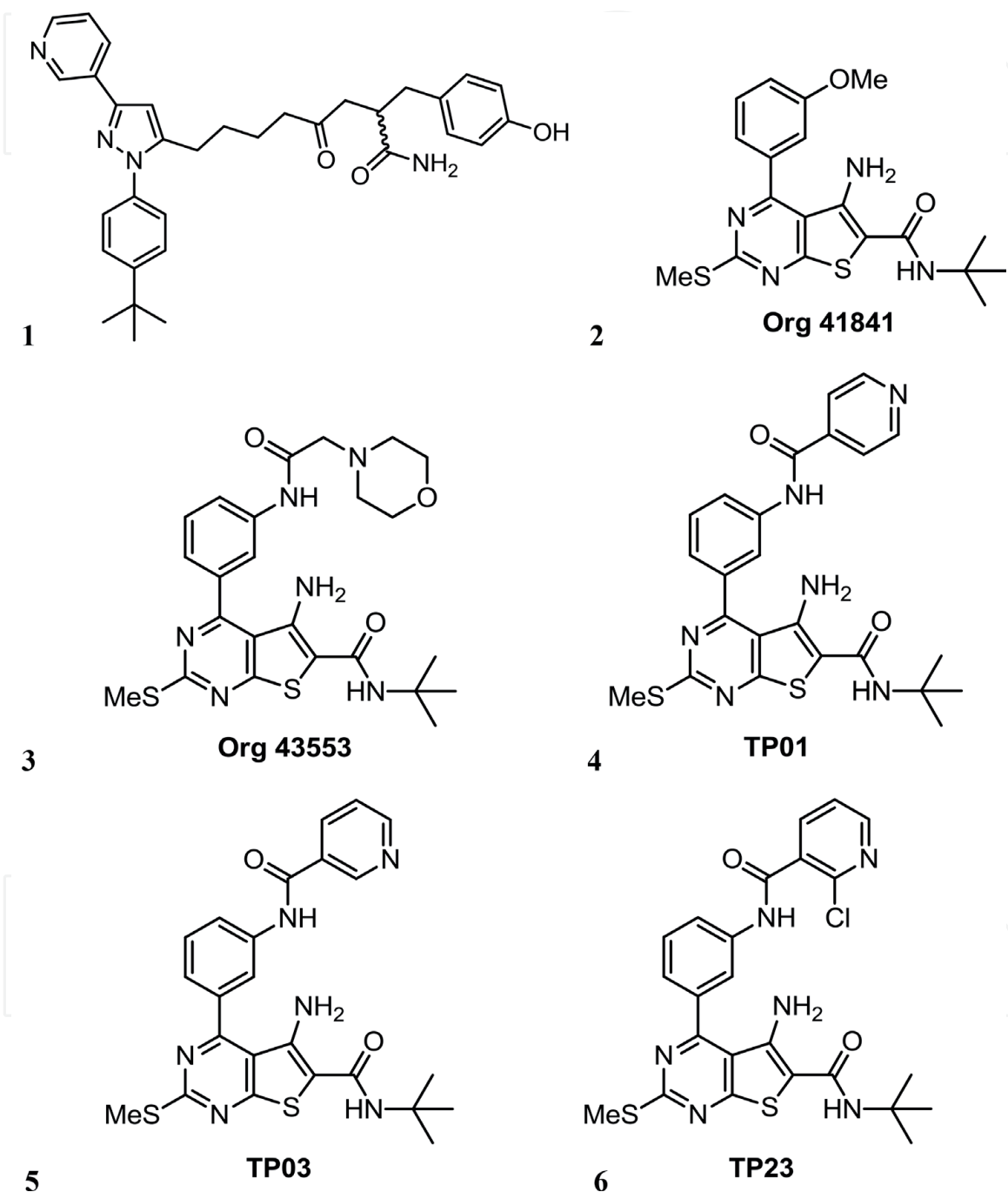


Figure 2.

The pyrazole- and thienopyrimidine-based low-molecular-weight agonists of LH receptor. (1) 8-(1-(4-(*tert*-butyl)phenyl)-3-(pyridin-3-yl)-1*H*-pyrazol-5-yl)-2-(4-hydroxybenzyl)-4-oxooctanamide [27]; (2) Compound Org 41,841, *N*-*tert*-butyl-5-amino-4-(3-methoxyphenyl)-2-(methylthio)thieno[2,3-*d*]pyrimidine-6-carboxamide [31]; (3) Compound Org 43,553, 5-amino-*N*-(*tert*-butyl)-2-(methylthio)-4-(3-(2-morpholinoacetamido)phenyl)thieno[2,3-*d*]pyrimidine-6-carboxamide [31]; (4) Compound TP01, 5-amino-*N*-(*tert*-butyl)-4-(3-(isonicotinamido)phenyl)-2-(methylthio)thieno[2,3-*d*]pyrimidine-6-carboxamide [32]; (5) Compound TP03, 5-amino-*N*-*tert*-butyl-2-(methylthio)-4-(3-(nicotinamido)phenyl)thieno[2,3-*d*]pyrimidine-6-carboxamide [33]; (6) Compound TP23, 5-amino-*N*-(*tert*-butyl)-4-(3-(2-chloronicotinamido)phenyl)-2-(methylthio)thieno[2,3-*d*]pyrimidine-6-carboxamide [34].

administered intraperitoneally to male rats, this compound stimulated the testosterone production in them [27]. However, the greatest success was achieved in the development of the thienopyrimidine-based agonists of LH receptor.

In 2002, as a result of screening of a large number of the organic compounds, the Dutch scientists from the Organon Company discovered the first compounds with activity of LH receptor agonists belonging to the thienopyrimidines [31]. The most effective among them were the compound Org 41,841 and its analogue Org 43,553 (**Figure 2**). Later, the pharmacological characteristics of Org 43,553 and the mechanisms of its action were studied, and this compound was considered as the “gold” standard for the allosteric agonists of LH receptor [35–39]. Based on Org 43,553 structure, we have developed and studied the series of the thienopyrimidine derivatives that with a high efficiency stimulated the AC activity and steroidogenesis in the Leydig cells in both the in vitro and in vivo conditions [33–35, 40–42]. The most active among these derivatives were the compounds TP01, TP03, and TP23 (**Figure 2**). The study of thienopyrimidine-based LMW agonists of LH receptor allowed identifying the mechanisms of their action and the pharmacological profile, which can be an advantage when using these compounds in the clinic.

Using the Org 41,841 and Org 43,553, the allosteric sites in the transmembrane channels of gonadotropins receptors and related to them thyroid-stimulating hormone (TSH) receptor were carried out. This allowed to detect the structural features of the active and inactive conformations of the serpentine domain of gonadotropins receptors, to identify the amino acid residues involved in the formation of their allosteric sites, and to decipher the mechanisms of signal transduction through these receptors [43, 44]. Based on the site-directed mutagenesis, the amino acid residues in the second extracellular loop (ECL2) and within the fifth and sixth transmembrane regions (TM5 and TM6) of TSH receptor, which form its allosteric site, were replaced with the corresponding amino acids of LH receptor, which made the allosteric site of TSH receptor similar to that in LH receptor. It was found that a single substitution, Leu⁵⁷⁰Phe, in the ECL2 of TSH receptor resulted in the Org 41,841 binding with a mutant receptor with the EC₅₀ of 800 nM, while the double substitutions, the Leu⁵⁷⁰Phe/Phe⁵⁸⁵Thr and Leu⁵⁷⁰Phe/Tyr⁶⁴³Phe, led to the Org 41,841 binding with the EC₅₀ of 1000 nM. These data indicate an important role of the residues Lyr⁵⁷⁰Phe, Phe⁵⁸⁵Thr, and Tyr⁶⁴³Phe in the formation of the allosteric site in LH receptor [43]. The simultaneous replacement of nine amino acids (Ile⁵⁶⁰Val and Leu⁵⁷⁰Phe in the ECL2; Prp⁵⁷⁷Thr, Ala⁵⁷⁹Ser, Leu⁵⁸⁰Gln, Ala⁵⁸¹Val, and Phe⁵⁸⁵Thr in the TM5; and Tyr⁶⁴³Phe and Ile⁶⁴⁸Ala in the TM6) that form the allosteric site of TSH receptor with the corresponding amino acids of LH receptor induced a high-affinity binding of Org 41,841 with mutant TSH receptor, similar to that of LH receptor. In the cells with expressed mutant TSH receptor, the AC stimulating effects induced by the Org 41,841 and TSH were similar [43]. It was also shown that the negatively charged Glu⁵⁰⁶ located in the TM3 of the LH and TSH receptors has a key role in specific interaction with Org 41,841, since the substitution of Glu⁵⁰⁶Ala inhibits both the Org 41,841 binding to mutant LH receptor and the AC stimulating effect of Org 41,841 [43, 44].

The allosteric LMW agonists stabilize any one active conformation and, thereby, selectively stimulate preferably one intracellular cascade. It was shown that the Org 43,553 (1–10 μM) stimulated the activity of PLCβ by 33–37%, which is less than 5% of the corresponding effect of LH [36]. Moreover, the Org 43,553 effectively stimulated the activity of AC and cAMP-dependent transcription factors at lower concentrations. Based on these data, a conclusion was made on the selectivity of stimulating influence of Org 43,553 on the AC signaling system, which is realized

through the G_s proteins and on the inefficiency of this compound in regard to the $G_{q/11}$ proteins and PLC β -dependent signaling [36].

Using the bacterial toxin-induced ADP ribosylation and the peptide strategy, we showed the selectivity of the thienopyrimidine derivative TP03, as a stimulator of the AC signaling system in the rat testicular and ovarian membranes [45]. In the membranes treated with cholera toxin that hyperactivates the G_s proteins and prevents the signal transduction through them, the stimulating effects of TP03 on the AC activity and the GTP binding were suppressed. At the same time, the treatment of the plasma membranes with pertussis toxin, which inhibits the G_i proteins, and their incubation with the peptide 349–359 of $G_{\alpha_{q/11}}$ -subunit, which leads to uncoupling of the LH receptor and $G_{q/11}$ proteins, did not affect the regulatory effects of TP03. Only at high concentrations (10–100 μ M), the TP03 effect on the GTP binding of $G_{q/11}$ proteins was detected but to a small extent. Under the same conditions, the regulatory effects of hCG were not specific for different types of G proteins [45]. It should be noted that the thienopyrimidine derivatives and the other allosteric ligands of LH receptor, which did not affect the AC activity and the steroidogenesis, are usually excluded from further research. However, they can activate G_s -independent signaling cascades, including the $G_{q/11}$ proteins and β -arrestins. As a consequence, these compounds may be of interest for studying the molecular mechanisms of the allosteric regulation of LH receptors and can be used in medicine.

The selectivity of signal transduction may have an important role in maintaining the tissue sensitivity to both the gonadotropins and LMW agonists, which was demonstrated by us in the case of TP03 [42]. Despite the fact that hCG and TP03 increased the testosterone levels during the 7-day treatment of male rats, a dynamics of this effect differed significantly. In long-term hCG treatment, an increase in the plasma testosterone concentration was the maximum on the first day, and then it decreased. At the same time, the steroidogenic effect of TP03, on the contrary, gradually increased, reaching a maximum on the seventh day of treatment. On the first day of treatment, TP03-induced increase in the testosterone concentration was 4 times lower than that in the case of hCG, while at the end of the experiment, the steroidogenic effects of hCG and TP03 were comparable [42]. One of the causes for this may be the specific changes in the LH receptor sensitivity to gonadotropins and thienopyrimidines, as well as the different mechanisms of their action on steroidogenesis.

A long-term administration of hCG to male rats leads to a significant decrease in the testicular expression of the *Lhr* gene encoding LH receptor, while a long-term administration of TP03 induces an increase in the *Lhr* gene expression, which is one of the factors maintaining the sensitivity of the Leydig cells to gonadotropins [42]. It should be noted that the gonadotropin resistance of the reproductive tissues is one of the urgent problems of the LH and hCG applications to treat the reproductive dysfunctions and in the assisted reproductive technologies [17, 46]. In our experiments, both the hCG and TP03 increased the expression of the *Star* gene, which encodes the steroidogenic acute regulatory protein (StAR) responsible for cholesterol transport into mitochondria, the rate-limiting stage of steroidogenesis, and gonadotropin in this regard was more active [42]. It was shown that the more the *Lhr* gene expression and the steroidogenic effect of the drug on testosterone production were decreased, the more the *Star* gene expression was increased. This may indicate a compensatory mechanism for increasing the *Star* gene expression in the conditions of the impaired gonadotropin signaling and the reduced PKA-induced stimulation of the StAR protein in the Leydig cells.

In the case of a long-term treatment of male rats with hCG, the intratesticular expression of the *Cyp11a1* gene encoding the C27 cholesterol side-chain cleavage cytochrome P450 (cytochrome P450_{sc}) that converts cholesterol to pregnenolone was significantly increased, and on the seventh day, the expression of the *Hsd3b* gene encoding the 3 β -hydroxysteroid dehydrogenase/ Δ 5–4 isomerase (3 β -HSD) that converts pregnenolone to progesterone was also increased. When the TP03 was used, there were no significant changes in the expression of the *Cyp11a1* and *Hsd3b* genes, which indicates a stable functioning of the steroidogenesis system in the conditions of a long-term treatment of animals with LMW agonist [42].

Along with the selectivity of the intracellular signaling induced by the thienopyrimidine derivatives, which identifies them as the selective bias agonists of LH receptor, their pharmacokinetic characteristics also contribute to the stability of the steroidogenic effect of these LMW agonists. When administered to rats, the Org 43,553 shows a half-life time of 3.4 hours, while the half-life time for hCG is 6.6 hours, indicating the more rapid degradation and excretion of LMW agonist than gonadotropin [37]. Reducing the half-life time for thienopyrimidines is of great practical importance, since it contributes to maintaining the tissue sensitivity to endogenous gonadotropins and, in addition, reduces the risk of ovarian hyperstimulation syndrome, a severe complication of gonadotropin-induced ovarian stimulation in the assisted reproductive technologies. Unlike gonadotropins, both the single and long-term treatments of female rats with Org 43,553 did not cause an increase in the ovarian diameter and the vascular permeability in the ovary and did not provoke the development of ovarian hyperstimulation syndrome [38]. Also, there were no signs of the ovarian hyperstimulation syndrome in women who received Org 43,553 orally at the doses from 25 to 900 mg. In 83% of women, the administration of Org 43,553 at a single dose of 300 mg caused the ovulation and the production of high-quality oocytes [47].

Since the orthostatic and allosteric sites in LH receptor do not overlap, the LMW allosteric agonists do not inhibit the specific LH and hCG binding [27, 35, 36]. Moreover, the AC stimulating effects of the LMW agonists and gonadotropins are additive, at least in a range of their concentrations lower than the EC₅₀ values [33, 35, 36, 41]. As shown by us, the steroidogenic effect of hCG in male rats pretreated with the TP03 was enhanced significantly, and this potentiating effect of TP03 was most pronounced at the low doses of hCG (Shpakov, Bakhtyukov and Derkach, unpublished data). This effect of thienopyrimidines can be due to their chaperone-like properties (**Figure 3**). It was shown that the Org 42,599, the trifluoroacetate salt of Org 43,553, restored the activity of the mutant LH receptors with the Ala⁵⁹³Pro and Ser⁶¹⁶Tyr replacements [48]. The incubation of the cells expressing these receptors with Org 42,599 led to an increase in the expression of the mutant receptors, the number of the receptors with the appropriate folding, and membrane topology and the receptor density on the cell surface. The chaperone-like properties of Org 42,599 are due to its ability to penetrate the plasma membrane and specifically bind to an allosteric site of intracellularly located LH receptors, which promotes their efficient translocation into the plasma membrane of the Leydig cells [48]. It should be emphasized that the LH receptors with the Ala⁵⁹³Pro and Ser⁶¹⁶Tyr mutations in the transmembrane regions are not capable of translocation into the cell surface and cannot be activated by gonadotropins. These mutations were found in patients with hypoplasia of the Leydig cells [49–52].

The allosteric sites of closely related GPCRs are known to be characterized by the variability of the primary structure and the three-dimensional organization, which in most cases makes the allosteric regulators more specific than the orthosteric regulators [26, 53]. In the case of the receptors of pituitary glycoprotein

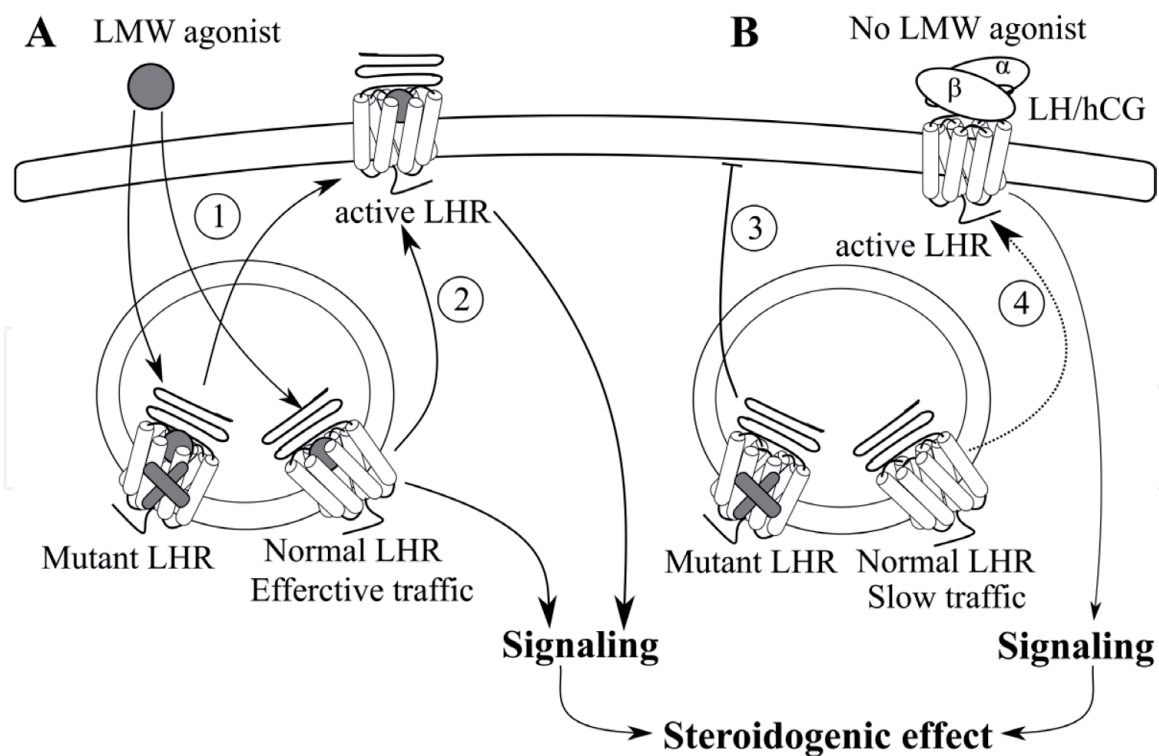


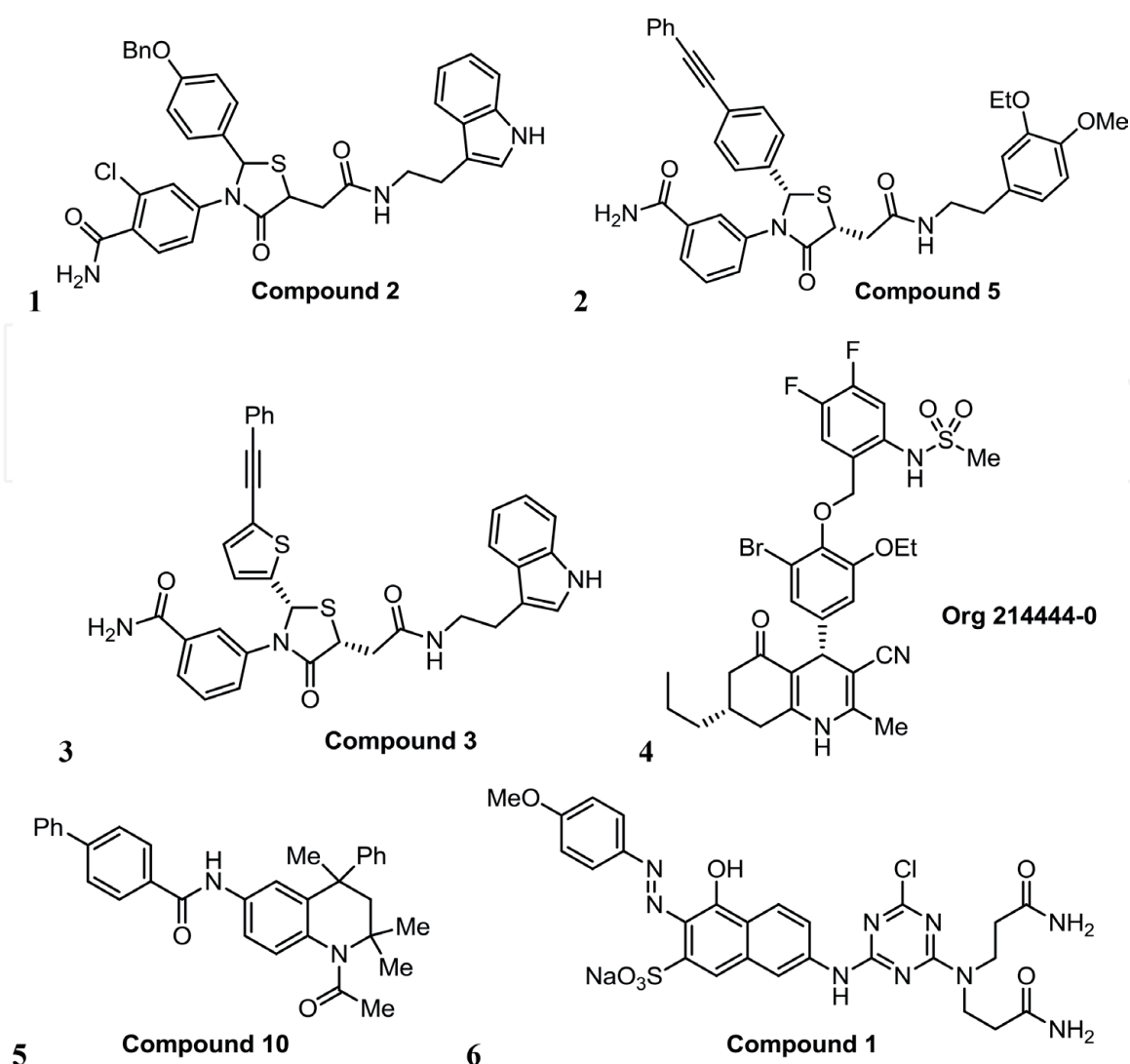
Figure 3.

The chaperone-like properties of the LMW allosteric agonists. (A) The LMW agonist, being a hydrophobic substance, penetrates the plasma membrane, interacts with normal LH receptors located in the intracellular vesicles, and ensures their effective translocation into the plasma membrane of Leydig cells (1). This effect can be realized in the case of the mutant LH receptors that are not capable of normal posttranslational processing and translocation into the membrane (2). As a result, the treatment with the LMW agonists leads to the preservation and even to an increase in the sensitivity of LH-competent cells to LH and hCG. B. In the absence of agonists, the mutant LH receptors are not able to translocate into the membrane (3), while the normal forms of the receptor are retained within the cell for a longer time (4). All this leads to a weakened response to gonadotropins with LH activity.

hormones, the specificity of LMW agonists to their allosteric site is not so high that it can be assumed to be due to the inverted position of the orthosteric and allosteric sites in these receptors as compared to other GPCRs belonging to class A [26]. At the same time, we and other authors showed that the Org 43,553, TP01, TP03, and TP23 have a very weak effect on the activity of the FSH and TSH receptors [36, 54]. When administered to male rats, the TP01, TP03, and TP23 did not affect the basal and thyroliberin-stimulated levels of thyroid hormones [54].

4. The low-molecular-weight ligands of the follicle-stimulating hormone receptor

The first LMW allosteric agonist of FSH receptor, a piperidine carboxamide, was developed in 2001. It stimulated the AC activity in the CHO cells expressing the FSH receptor (EC_{50} , 3.9 nM) but was not active in the in vivo conditions [55]. A search for new LMW ligands of FSH receptor revealed a large number of the compounds with different pharmacological activity [39, 56–58]. They belong to different classes of the organic compounds, such as the thiazolidines [59–62], substituted γ -lactams [63], diketopiperazines [64, 65], *N*-alkylated sulfonyl piperazines [66], tetrahydroquinolines [67], hexahydroquinolines [68, 69], thienopyrimidines [70], and benzamides [69]. Among the developed LMW ligands, the thiazolidines, hexa- and tetrahydroquinolines, and benzamides with activity of the full and inverse agonists and the neutral antagonists of FSH receptor are of the greatest interest.

**Figure 4.**

The low-molecular-weight allosteric agonists and antagonists of the FSH receptor. (1) Compound 2, 4-(5-(2-((2-(1H-indol-3-yl)ethyl)amino)-2-oxoethyl)-2-(4-(benzyloxy)phenyl)-4-oxothiazolidin-3-yl)-2-chlorobenzamide [59]; (2) Compound 5, 3-(2S,5R)-5-(2-((3-ethoxy-4-methoxyphenethyl)amino)-2-oxoethyl)-4-oxo-2-(4-(phenylethynyl)phenyl)thiazolidin-3-yl)benzamide [61]; (3) Compound 3, 3-(2S,5R)-5-(2-((2-(1H-indol-3-yl)ethyl)amino)-2-oxoethyl)-4-oxo-2-(5-(phenylethynyl)thiophen-2-yl)thiazolidin-3-yl)benzamide [62]; (4) Compound Org214444-0, N-(2-((2-bromo-4-((4R,7S)-3-cyano-2-methyl-5-oxo-7-propyl-1,4,5,6,7,8-hexahydroquinolin-4-yl)-6-ethoxyphenoxy)methyl)-4,5-difluorophenyl)methanesulfonamide [68]; (5) Compound 10, N-(1-acetyl-2,2,4-trimethyl-4-phenyl-1,2,3,4-tetrahydroquinolin-6-yl)-[1,1'-biphenyl]-4-carboxamide [67]; (6) Compound 1, 7-[4-[bis-(2-carbamoyl-ethyl)-amino]-6-chloro-(1,3,5)-triazin-2-ylamino]-4-hydroxy-3-(4-methoxy-phenylazo)-naphthalene}-2-sulfonic acid sodium salt [71].

In 2004, compound 2 belonging to the thiazolidines was developed (**Figure 4**). It suppressed FSH-induced stimulating effects and, by its pharmacological profile, was classified as an inverse agonist [59]. Based on its structure, the thiazolidine derivatives with the activity of the full agonists were developed [60, 61, 63], including highly active compound 5 (**Figure 4**). This compound stimulated the cAMP-dependent cascades and the steroidogenesis in the cell cultures and in the in vivo conditions induced the development of preovulatory follicles and stimulated the ovulation in immature female rats [61, 72]. Compound 5 was able to enhance the stimulating effects of low-dose FSH, acting as the PAM for FSH receptor. Another thiazolidine derivative, compound 3 (**Figure 4**), at the low concentrations activated the G_s proteins and stimulated the cAMP-dependent cascades in the cells expressing the FSH receptor, functioning as a full agonist. At the same, at the high concentrations, compound 3 inhibited FSH-induced activation of G_s proteins and stimulated the G_i proteins, reducing the activity of AC and cAMP-dependent transcription factors, functioning as the NAM [62, 73].

In 2006, the compound Org214444-0, a derivative of 4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinolines (**Figure 4**), was developed, which in the absence of FSH caused the AC activation in CHO cells expressing the FSH receptor (EC_{50} about 1 nM) and stimulated the steroidogenesis in the human and rat granulosa cells [68, 69]. Moreover, Org214444-0 increased the FSH affinity to receptor and the efficiency of FSH-induced AC stimulation, which makes it possible to identify this compound as ago-PAM. Oral administration of Org214444-0 led to the stimulation of the folliculogenesis and induced the ovulation in mature female rats, indicating the stability and effective absorption of Org214444-0 in the gastrointestinal tract [69].

Compound 10, a tetrahydroquinoline derivative (**Figure 4**), with a high efficiency inhibited FSH-induced follicular growth and ovulation in mice [67]. There is reason to believe that compound 10 prevents the functional interaction between the extracellular and serpentine domains of FSH receptor and, thereby, disrupts the signal transduction from the ligand-binding site located within an ectodomain to the intracellular regions of receptor that are involved in the interaction with the G_s proteins. This characterizes this compound as NAM for FSH receptor [67]. Additionally, it has been shown that the 7-{4-[bis-(2-carbamoyl-ethyl)-amino]-6-chloro-(1,3,5)-triazin-2-ylamino)-4-hydroxy-3-(4-methoxy-phenylazo)-naphthalene}-2-sulfonic acid (compound 1) (**Figure 4**), also belonging to NAM for FSH receptor, in a dose-dependent manner reduced the specific FSH binding to the receptor, suppressed the stimulating effects of FSH on the AC activity and steroidogenesis, and in the *in vivo* conditions prevented FSH-induced ovulation in female rats [71, 74].

Among the benzamide derivatives, the most active were the compounds ADX61623, ADX68692, and ADX68693, which demonstrated the activity of allosteric antagonists of FSH receptor [75, 76]. These compounds had an unusual pharmacological profile, which, as may be supposed, was due to the complexity of their effect on the FSH-stimulated signaling in the target cells. In the *in vitro* conditions, the *N*-(4-(2-cyanopropane-2-yl)phenyl)-3,4-dimethoxybenzamide (ADX61623) at the low concentrations inhibited FSH-induced production of cAMP and progesterone in follicular cells, while at the high concentrations, it increased the production of estradiol [75]. Of the three benzamide derivatives, only the ADX68692 was active when administered orally and subcutaneously to mature female rats, dysregulating the sexual cycle and reducing the number of matured oocytes [76]. However, it should be noted that the ADX68692 was able to modulate the activity of LH receptor, enhancing the production of progesterone and reducing the synthesis of testosterone in the rat Leydig cells [77].

In conclusion, it should be noted that despite the large number of the investigated allosteric ligands of FSH receptor, they are not yet used in the clinic due to the many unresolved problems with their bioavailability, the mechanisms of action, and possible undesirable effects [57, 58]. However, the limitations with the use of commercial FSH drugs in the clinic and the need to develop the selective FSH receptor inhibitors are a good stimulus to further development of the LMW ligands of FSH receptor and their implementation into clinical practice.

5. Conclusion and future perspectives

Summing up the current results in the development and study of the LMW allosteric ligands of the LH and FSH receptors, it is necessary to focus on the following advantages, which are important for their application for reproduction and molecular and clinical endocrinology, including the assisted reproductive technologies.

- i. The LMW allosteric agonists of the gonadotropins receptors do not compete with the gonadotropins for the binding sites and, thus, do not suppress the effects of LH, hCG, and FSH, and in some cases they enhance them, acting as PAM or ago-PAM. The inhibition of the stimulating effect of gonadotropins by the LMW allosteric inverse agonists and NAMs is due to their allosteric effects, but not the result of the competition for receptor binding sites.
- ii. The LMW ligands of the LH and FSH receptors are characterized by the selectivity for intracellular signaling cascades, functioning as the bias ligands, which allows predicting and determining the functional response of cells to their action and prevents a number of undesirable side effects that are detected when gonadotropins are used.
- iii. Since the LMW agonists are selective, they, unlike gonadotropins, have a little effect on the β -arrestin signaling pathways responsible for downregulation of the receptors. As a result, under conditions of treatment with the LMW allosteric agonists, the sensitivity of the tissues to endogenous gonadotropins is preserved, which makes it possible to use the long-term courses of LMW agonists as well as to use them with the gonadotropins.
- iv. The LMW allosteric ligands of the LH and FSH receptors can be active not only with their parenteral routes of administration but also with their oral delivery, since they are stable in the gastrointestinal tract and are well absorbed by intestinal cells.
- v. The LMW agonists have chaperone-like properties in relation to the LH and FSH receptors, preventing their intracellular degradation and increasing their translocation to the plasma membrane. In this regard, the LMW agonists can be used to enhance the response of the reproductive system to the gonadotropins in the case of the mutant LH and FSH receptors that are not capable of translocation as well as in the conditions of the metabolic, inflammatory, and autoimmune disorders inducing the impaired posttranslational processing of these receptors. It should be noted that the mutations and polymorphisms in the gonadotropin receptors lead to a decrease in the sensitivity of the testes and ovaries to gonadotropins [78, 79]. In the assisted reproductive technologies, they reduce the response of the ovaries to the gonadotropin stimulation, which leads to the impaired folliculogenesis, the reduced output of high-quality oocytes, and the deterioration of the development and implantation of the embryo [78, 80]. Since both gonadotropins, LH and FSH, play an important role in the development and maturation of the follicles and oocytes, the polymorphisms in the LH and FSH receptors can be the main causes of an impairment of folliculogenesis and oogenesis, including their late stages [79, 81, 82].

Despite the advantages listed above, the LMW allosteric ligands of the LH and FSH receptors have not yet found use in the clinic. The main reason is insufficient knowledge of the pharmacokinetics and the distribution of these compounds in the body, as well as the problems with the development of their dosage forms, especially since most of these compounds are highly hydrophobic and dissolve in DMSO. The attempts to reduce the hydrophobicity of LMW ligands by modifying their structure lead to the partial or complete loss of their specific activity, due to their reduced ability of penetration into the transmembrane channel of

the gonadotropins receptors. The most promising approach to solve this problem is to use the solubilizing agents that can increase the water solubility of these compounds.

Thus, the development of the LMW allosteric ligands of the gonadotropin receptors, which have a high specificity in regulating certain signaling pathways and effector systems in the testicular and ovarian cells, opens up a promising way to create a new generation of highly selective drugs that can be used to treat and prevent the reproductive disorders and in assisted reproductive technologies, both separately and in combination with gonadotropins and other regulators of the hypothalamic-pituitary-gonadal axis.

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Disclosure

Conflicts of interest are absent.

Abbreviations

AC	adenylyl cyclase
ECL ₂	second extracellular loop
FSH	follicle-stimulating hormone
GPCR	G protein-coupled receptor
hCG	human chorionic gonadotropin
LH	luteinizing hormone
LMW ligand	low-molecular-weight ligand
MAPK	mitogen-activated protein kinases
NAM	negative allosteric modulator
PAM	positive allosteric modulator
PLCβ	phosphoinositide-specific phospholipase Cβ
TSH	thyroid-stimulating hormone

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Author details

Alexander O. Shpakov^{1*}, Kira V. Derkach¹, Andrey A. Bakhtyukov¹
and Dmitry V. Dar'in²

1 Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian
Academy of Sciences, St. Petersburg, Russia

2 Institute of Chemistry, St. Petersburg State University, Russia

*Address all correspondence to: alex_shpakov@list.ru

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References

- [1] De Leo V, Musacchio MC, Di Sabatino A, Tosti C, Morgante G, Petraglia F. Present and future of recombinant gonadotropins in reproductive medicine. *Current Pharmaceutical Biotechnology*. 2012;**13**(3):379-391. DOI: 10.2174/138920112799361918
- [2] Ezcurra D, Humaidan P. A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. *Reproductive Biology and Endocrinology*. 2014;**12**:95. DOI: 10.1186/1477-7827-12-95
- [3] Plant TM. 60 years of neuroendocrinology: The hypothalamo-pituitary-gonadal axis. *The Journal of Endocrinology*. 2015;**226**:T41-T54. DOI: 10.1530/JOE-15-0113
- [4] Ulloa-Aguirre A, Lira-Albarrán S. Clinical applications of gonadotropins in the male. *Progress in Molecular Biology and Translational Science*. 2016;**143**:121-174. DOI: 10.1016/bs.pmbts.2016.08.003
- [5] Levi Setti PE, Alviggi C, Colombo GL, Pisanelli C, Ripellino C, Longobardi S, et al. Human recombinant follicle stimulating hormone (rFSH) compared to urinary human menopausal gonadotropin (HMG) for ovarian stimulation in assisted reproduction: A literature review and cost evaluation. *Journal of Endocrinological Investigation*. 2015;**38**(5):497-503. DOI: 10.1007/s40618-014-0204-4
- [6] Fournier T. Human chorionic gonadotropin: Different glycoforms and biological activity depending on its source of production. *Annales de Endocrinologie*. 2016;**77**(2):75-81. DOI: 10.1016/j.ando.2016.04.012
- [7] Van Dorsselaer A, Carapito C, Delalande F, Schaeffer-Reiss C, Thierse D, Diemer H, et al. Detection of prion protein in urine-derived injectable fertility products by a targeted proteomic approach. *PLoS One*. 2011;**6**:e17815. DOI: 10.1371/journal.pone.0017815
- [8] Cole LA. hCG, the wonder of today's science. *Reproductive Biology and Endocrinology*. 2012;**10**:24. DOI: 10.1186/1477-7827-10-24
- [9] Theofanakis C, Drakakis P, Besharat A, Loutradis D. Human chorionic gonadotropin: The pregnancy hormone and more. *International Journal of Molecular Sciences*. 2017;**18**(5):pii: E1059. DOI: 10.3390/ijms18051059
- [10] Bousfield GR, Dias JA. Synthesis and secretion of gonadotropins including structure-function correlates. *Reviews in Endocrine and Metabolic Disorders*. 2011;**12**(4):289-302. DOI: 10.1007/s11154-011-9191-3
- [11] Ji I, Lee C, Jeoung M, Koo Y, Sievert GA, Ji TH. Trans-activation of mutant follicle-stimulating hormone receptors selectively generates only one of two hormone signals. *Molecular Endocrinology*. 2004;**18**:968-978. DOI: 10.1210/me.2003-0443
- [12] Puett D, Li Y, DeMars G, Angelova K, Fanelli F. A functional transmembrane complex: The luteinizing hormone receptor with bound ligand and G protein. *Molecular and Cellular Endocrinology*. 2007;**260-262**:126-136. DOI: 10.1016/j.mce.2006.05.009
- [13] Puett D, Angelova K, da Costa MR, Warrenfeltz SW, Fanelli F. The luteinizing hormone receptor: Insights into structure-function relationships and hormone-receptor-mediated changes in gene expression in ovarian cancer cells. *Molecular and Cellular*

Endocrinology. 2010;**329**(1-2):47-55. DOI: 10.1016/j.mce.2010.04.025

[14] Angelova K, Felling A, Lee M, Patel M, Puett D, Fanelli F. Conserved amino acids participate in the structure networks deputed to intramolecular communication in the lutropin receptor. Cellular and Molecular Life Sciences. 2011;**68**(7):1227-1239. DOI: 10.1007/s00018-010-0519-z

[15] Ulloa-Aguirre A, Dias JA, Bousfield G, Huhtaniemi I, Reiter E. Trafficking of the follitropin receptor. Methods in Enzymology. 2013;**521**:17-45. DOI: 10.1016/B978-0-12-391862-8.00002-8

[16] De Pascali F, Tréfier A, Landomiel F, Bozon V, Bruneau G, Yvinec R, et al. Follicle-stimulating hormone receptor: Advances and remaining challenges. International Review of Cell and Molecular Biology. 2018;**338**:1-58. DOI: 10.1016/bs.ircmb.2018.02.001

[17] Riccetti L, De Pascali F, Gilioli L, Potì F, Giva LB, Marino M, et al. Human LH and hCG stimulate differently the early signalling pathways but result in equal testosterone synthesis in mouse Leydig cells *in vitro*. Reproductive Biology and Endocrinology. 2017;**15**(1):2. DOI: 10.1186/s12958-016-0224-3

[18] Riccetti L, Yvinec R, Klett D, Gallay N, Combarnous Y, Reiter E, et al. Human luteinizing hormone and chorionic gonadotropin display biased agonism at the LH and LH/CG receptors. Scientific Reports. 2017;**7**(1):940. DOI: 10.1038/s41598-017-01078-8

[19] Landomiel F, De Pascali F, Raynaud P, Jean-Alphonse F, Yvinec R, Pellissier LP, et al. Biased signaling and allosteric modulation at the FSHR. Frontiers in Endocrinology. 2019;**10**:148. DOI: 10.3389/fendo.2019.00148

[20] Kara E, Crépieux P, Gauthier C, Martinat N, Piketty V, Guillou F, et al. A phosphorylation cluster of five serine and threonine residues in the C-terminus of the follicle-stimulating hormone receptor is important for desensitization but not for beta-arrestin-mediated ERK activation. Molecular Endocrinology. 2006;**20**(11):3014-3026. DOI: 10.1210/me.2006-0098

[21] Reiter E, Ahn S, Shukla AK, Lefkowitz RJ. Molecular mechanism of beta-arrestin-biased agonism at seven-transmembrane receptors. Annual Review of Pharmacology and Toxicology. 2012;**52**:179-197. DOI: 10.1146/annurev.pharmtox.010909.105800

[22] Casarini L, Reiter E, Simoni M. β -Arrestins regulate gonadotropin receptor-mediated cell proliferation and apoptosis by controlling different FSHR or LHCGR intracellular signaling in the hGL5 cell line. Molecular and Cellular Endocrinology. 2016;**437**:11-21. DOI: 10.1016/j.mce.2016.08.005

[23] Ulloa-Aguirre A, Crépieux P, Poupon A, Maurel MC, Reiter E. Novel pathways in gonadotropin receptor signaling and biased agonism. Reviews in Endocrine and Metabolic Disorders. 2011;**12**(4):259-274. DOI: 10.1007/s11154-011-9176-2

[24] Fournier T, Guibourdenche J, Evain-Brion D. Review: hCGs—Different sources of production, different glycoforms and functions. Placenta. 2015;**36**(Suppl 1):S60-S65. DOI: 10.1016/j.placenta.2015.02.002

[25] Nwabuobi C, Arlier S, Schatz F, Guzeloglu-Kayisli O, Lockwood CJ, Kayisli UA. hCG: Biological functions and clinical applications. International Journal of Molecular Sciences. 2017;**18**(10):pii: E2037. DOI: 10.3390/ijms18102037

[26] van der Westhuizen ET, Valant C, Sexton PM, Christopoulos A.

- Endogenous allosteric modulators of G protein-coupled receptors. *The Journal of Pharmacology and Experimental Therapeutics*. 2015;**353**:246-260. DOI: 10.1124/jpet.114.221606
- [27] Jorand-Lebrun C, Brondyk B, Lin J, Magar S, Murray R, Reddy A, et al. Identification, synthesis, and biological evaluation of novel pyrazoles as low molecular weight luteinizing hormone receptor agonists. *Bioorganic and Medicinal Chemistry Letters*. 2007;**17**(7):2080-2085. DOI: 10.1016/j.bmcl.2006.12.062
- [28] Heitman LH, Ijzerman AP. G protein-coupled receptors of the hypothalamic-pituitary-gonadal axis: A case for GnRh, LH, FSH, and GPR54 receptor ligands. *Medicinal Research Reviews*. 2008;**28**:975-1011. DOI: 10.1002/med.20129
- [29] Heitman LH, Narlawar R, de Vries H, Willemsen MN, Wolfram D, Brussee J, et al. Substituted terphenyl compounds as the first class of low molecular weight allosteric inhibitors of the luteinizing hormone receptor. *Journal of Medicinal Chemistry*. 2009;**52**:2036-2042. DOI: 10.1021/jm801561h
- [30] Heitman LH, Kleinau G, Brussee J, Krause G, Ijzerman AP. Determination of different putative allosteric binding pockets at the lutropin receptor by using diverse drug-like low molecular weight ligands. *Molecular and Cellular Endocrinology*. 2012;**351**(2):326-336. DOI: 10.1016/j.mce.2012.01.010
- [31] van Straten NC, Schoonus-Gerritsma GG, van Someren RG, Draaijer J, Adang AE, Timmers CM, et al. The first orally active low molecular weight agonists for the LH receptor: Thienopyr(im)idines with therapeutic potential for ovulation induction. *Chembiochem: A European Journal of Chemical Biology*. 2002;**3**(10):1023-1026. DOI: 10.1002/1439-7633(20021004)3:10<1023::AID-CBIC1023>3.0.CO;2-9
- [32] Shpakov AO, Dar'in DV, Derkach KV, Lobanov PS. The stimulating influence of thieno pyrimidine compounds on the adenylyl cyclase systems in the rat testes. *Doklady Biochemistry and Biophysics*. 2014;**456**:104-107. DOI: 10.1134/S1607672914030065
- [33] Derkach KV, Dar'in DV, Bakhtyukov AA, Lobanov PS, Shpakov AO. In vitro and in vivo studies of functional activity of new low molecular weight agonists of the luteinizing hormone receptor. *Biochemistry (Moscow). Supplement Series A: Membrane and Cell Biology*. 2016;**10**(4):294-300. DOI: 10.1134/S1990747816030132
- [34] Derkach KV, Legkodukh AS, Dar'in DV, Shpakov AO. The stimulating effect of thienopyrimidines structurally similar to Org 43553 on adenylyl cyclase activity in the testes and on testosterone production in male rats. *Cell and Tissue Biology*. 2017;**11**(1):73-80. DOI: 10.1134/S1990519X17010035
- [35] Heitman LH, Oosterom J, Bongers KM, Timmers CM, Wiegerinck PHG, Ijzerman AP. [³H]org 43553, the first low-molecular-weight agonistic and allosteric radioligand for the human luteinizing hormone receptor. *Molecular Pharmacology*. 2008;**73**(2):518-524. DOI: 10.1124/mol.107.039875
- [36] van Koppen CJ, Zaman GJ, Timmers CM, Kelder J, Mosselman S, van de Lagemaat R, et al. A signaling-selective, nanomolar potent allosteric low molecular weight agonist for the human luteinizing hormone receptor. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2008;**378**(5):503-514. DOI: 10.1007/s00210-008-0318-3

- [37] van de Lagemaat R, Timmers CM, Kelder J, van Koppen C, Mosselman S, Hanssen RG. Induction of ovulation by a potent, orally active, low molecular weight agonist (Org 43553) of the luteinizing hormone receptor. *Human Reproduction*. 2009;**24**(3):640-648. DOI: 10.1093/humrep/den412
- [38] van de Lagemaat R, Raafs BC, van Koppen C, Timmers CM, Mulders SM, Hanssen RG. Prevention of the onset of ovarian hyperstimulation syndrome (OHSS) in the rat after ovulation induction with a low molecular weight agonist of the LH receptor compared with hCG and rec-LH. *Endocrinology*. 2011;**152**(11):4350-4357. DOI: 10.1210/en.2011-1077
- [39] van Straten NC, Timmers CM. Non-peptide ligands for the gonadotropin receptors. *Annual Reports in Medicinal Chemistry*. 2009;**44**:171-188. DOI: 10.1016/S0065-7743(09)04408-X
- [40] Derkach KV, Dar'in DV, Lobanov PS, Shpakov AO. Intratesticular, intraperitoneal, and oral administration of thienopyrimidine derivatives increases the testosterone level in male rats. *Doklady Biological Sciences*. 2014;**459**(1):326-329. DOI: 10.1134/S0012496614060040
- [41] Shpakov AO, Derkach KV, Dar'in DV, Lobanov PS. Activation of adenylyl cyclase by thienopyrimidine derivatives in rat testes and ovaries. *Cell and Tissue Biology*. 2014;**8**(5):400-406. DOI: 10.1134/S1990519X14050071
- [42] Bakhtyukov AA, Derkach KV, Dar'in DV, Shpakov AO. Conservation of steroidogenic effect of the low-molecular-weight agonist of luteinizing hormone receptor in the course of its long-term administration to male rats. *Doklady Biochemistry and Biophysics*. 2019;**484**:78-81. DOI: 10.1134/S1607672919 010216
- [43] Jäschke H, Neumann S, Moore S, Thomas CJ, Colson AO, Costanzi S, et al. A low molecular weight agonist signals by binding to the transmembrane domain of thyroid-stimulating hormone receptor (TSHR) and luteinizing hormone/chorionic gonadotropin receptor (LHCGR). *The Journal of Biological Chemistry*. 2006;**281**(15):9841-9844. DOI: 10.1074/jbc.C600014200
- [44] Moore S, Jaeschke H, Kleinau G, Neumann S, Costanzi S, Jiang JK, et al. Evaluation of small-molecule modulators of the luteinizing hormone/choriogonadotropin and thyroid stimulating hormone receptors: Structure-activity relationships and selective binding patterns. *Journal of Medicinal Chemistry*. 2006;**49**(13):3888-3896. DOI: 10.1021/jm060247s
- [45] Derkach KV, Bakhtyukov AA, Shpakov AA, Dar'in DV, Shpakov AO. Specificity of heterotrimeric G protein regulation by human chorionic gonadotropin and low-molecular agonist of luteinizing hormone receptor. *Cell and Tissue Biology*. 2017;**11**(6):475-482. DOI: 10.1134/S1990519X17060037
- [46] Banker M, Garcia-Velasco JA. Revisiting ovarian hyper stimulation syndrome: Towards OHSS free clinic. *Journal of Human Reproductive Sciences*. 2015;**8**:13-17. DOI: 10.4103/0974-1208.153120
- [47] Gerrits M, Mannaerts B, Kramer H, Addo S, Hanssen R. First evidence of ovulation induced by oral LH agonists in healthy female volunteers of reproductive age. *The Journal of Clinical Endocrinology and Metabolism*. 2013;**98**:1558-1566. DOI: 10.1210/jc.2012-3404
- [48] Newton CL, Whay AM, McArdle CA, Zhang M, van Koppen CJ, van de Lagemaat R, et al. Rescue of expression and signaling of human luteinizing hormone G protein-coupled

receptor mutants with an allosterically binding small-molecule agonist. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**(17):7172-7176. DOI: 10.1073/pnas.1015723108

[49] Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, et al. Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nature Genetics*. 1995;**9**(2):160-164. DOI: 10.1038/ng0295-160

[50] Latronico AC, Anasti J, Arnhold IJ, Rapaport R, Mendonca BB, Bloise W, et al. Brief report: Testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. *The New England journal of medicine*. 1996;**334**(8):507-512. DOI: 10.1056/NEJM199602223340805

[51] Mizrachi D, Segaloff DL. Intracellularly located misfolded glycoprotein hormone receptors associate with different chaperone proteins than their cognate wild-type receptors. *Molecular Endocrinology*. 2004;**18**(7):1768-1777. DOI: 10.1210/me.2003-0406

[52] Ulloa-Aguirre A, Zariñán T, Dias JA, Conn PM. Mutations in G protein-coupled receptors that impact receptor trafficking and reproductive function. *Molecular and Cellular Endocrinology*. 2014;**382**(1):411-423. DOI: 10.1016/j.mce.2013.06.024

[53] Lindsley CW, Emmitte KA, Hopkins CR, Bridges TM, Gregory KJ, Niswender CM, et al. Practical strategies and concepts in GPCR allosteric modulator discovery: Recent advances with metabotropic glutamate receptors. *Chemical Reviews*. 2016;**116**(11):6707-6741. DOI: 10.1021/acs.chemrev.5b00656

[54] Bakhtyukov AA, Derkach KV, Dar'in DV, Shpakov AO. Thienopyrimidine

derivatives specifically activate testicular steroidogenesis but do not affect thyroid functions. *Journal of Evolutionary Biochemistry and Physiology*. 2019;**55**(1):30-39. DOI: 10.1134/S0022093019010046

[55] El Tayer N, Reddy A, Buckler D. Applied Research Systems ARS Holding NA, Assignee FSH Mimetics for the Treatment of Infertility. Unites States Patent US 6,235,755; 2001

[56] Nataraja SG, Yu HN, Palmer SS. Discovery and development of small molecule allosteric modulators of glycoprotein hormone receptors. *Frontiers in Endocrinology*. 2015;**6**:142. DOI: 10.3389/fendo.2015.00142

[57] Anderson RC, Newton CL, Anderson RA, Millar RP. Gonadotropins and their analogs: Current and potential clinical applications. *Endocrine Reviews*. 2018;**39**(6):911-937. DOI: 10.1210/er.2018-00052

[58] Anderson RC, Newton CL, Millar RP. Small molecule follicle-stimulating hormone receptor agonists and antagonists. *Frontiers in Endocrinology*. 2019;**9**:757. DOI: 10.3389/fendo.2018.00757

[59] Maclean D, Holden F, Davis AM, Scheuerman RA, Yanofsky S, Holmes CP, et al. Agonists of the follicle stimulating hormone receptor from an encoded thiazolidinone library. *Journal of Combinatorial Chemistry*. 2004;**6**(2):196-206. DOI: 10.1021/cc0300154

[60] Wrobel J, Jetter J, Kao W, Rogers J, Di L, Chi J, et al. 5-alkylated thiazolidinones as follicle-stimulating hormone (FSH) receptor agonists. *Bioorganic and Medicinal Chemistry*. 2006;**14**(16):5729-5741. DOI: 10.1016/j.bmc.2006.04.012

[61] Yanofsky SD, Shen ES, Holden F, Whitehorn E, Aguilar B, Tate E, et al.

Allosteric activation of the follicle-stimulating hormone (FSH) receptor by selective, nonpeptide agonists. *The Journal of Biological Chemistry*. 2006;**281**(19):13226-13233. DOI: 10.1074/jbc.M600601200

[62] Arey BJ. Allosteric modulators of glycoprotein hormone receptors: Discovery and therapeutic potential. *Endocrine*. 2008;**34**:1-10. DOI: 10.1007/s12020-008-9098-2

[63] Pelletier JC, Rogers J, Wrobel J, Perez MC, Shen ES. Preparation of highly substituted gamma-lactam follicle stimulating hormone receptor agonists. *Bioorganic and Medicinal Chemistry*. 2005;**13**:5986-5995. DOI: 10.1016/j.bmc.2005.07.025

[64] Guo T, Adang AE, Dolle RE, Dong G, Fitzpatrick D, Geng P, et al. Small molecule biaryl FSH receptor agonists. Part 1: Lead discovery via encoded combinatorial synthesis. *Bioorganic and Medicinal Chemistry Letters*. 2004;**14**(7):1713-1716. DOI: 10.1016/j.bmcl.2004.01.043

[65] Guo T, Adang AE, Dong G, Fitzpatrick D, Geng P, Ho KK, et al. Small molecule biaryl FSH receptor agonists. Part 2: Lead optimization via parallel synthesis. *Bioorganic and Medicinal Chemistry Letters*. 2004;**14**(7):1717-1720. DOI: 10.1016/j.bmcl.2004.01.043

[66] Magar S, Goutopoulos A, Liao Y, Schwarz M, Russell TJ. Piperazine Derivatives and Methods of Use. Patent WO2004031182A1; 2002

[67] Van Straten NC, van Berkel TH, Demont DR, Karstens WJ, Merks R, Oosterom J, et al. Identification of substituted 6-amino-4-phenyltetrahydroquinoline derivatives: Potent antagonists for the follicle-stimulating hormone receptor. *Journal of Medicinal Chemistry*.

2005;**48**(6):1697-1700. DOI: 10.1021/jm049676l

[68] Grima Poveda PM, Karstens Willem FJ, Timmers CM. Inventors, NV Organon, Assignee 4-Phenyl-5-Oxo-1,4,5,6,7,8-Hexahydroquinoline Derivatives for the Treatment of Infertility. United States patent US 8,022,218; 2006

[69] van Koppen CJ, Verboost PM, van de Lagemaat R, Karstens WJ, Loozen HJ, van Achterberg TA, et al. Signaling of an allosteric, nanomolar potent, low molecular weight agonist for the follicle-stimulating hormone receptor. *Biochemical Pharmacology*. 2013;**85**(8):1162-1170. DOI: 10.1016/j.bcp.2013.02.001

[70] Hanssen RGJM, Timmers CM. Thieno[2,3-d]pyrimidines with combined LH and FSH agonistic activity. World Patent WO2003020726. 2003

[71] Arey BJ, Deecher DC, Shen ES, Stevis PE, Meade EH, Wrobel J, et al. Identification and characterization of a selective, nonpeptide follicle-stimulating hormone receptor antagonist. *Endocrinology*. 2002;**143**:3822-3829

[72] Sriraman V, Denis D, de Matos D, Yu H, Palmer S, Nataraja S. Investigation of a thiazolidinone derivative as an allosteric modulator of follicle stimulating hormone receptor: Evidence for its ability to support follicular development and ovulation. *Biochemical Pharmacology*. 2014;**89**(2):266-275. DOI: 10.1016/j.bcp.2014.02.023

[73] Zoenen M, Urizar E, Swillens S, Vassart G, Costagliola S. Evidence for activity-regulated hormone-binding cooperativity across glycoprotein hormone receptor homomers. *Nature Communications*. 2012;**3**:1007. DOI: 10.1038/ncomms1991

- [74] Wrobel J, Green D, Jetter J, Kao W, Rogers J, Pérez MC, et al. Synthesis of (bis)sulfonic acid, (bis)benzamides as follicle-stimulating hormone (FSH) antagonists. *Bioorganic and Medicinal Chemistry*. 2002;**10**(3):639-656. DOI: 10.1016/S0968-0896(01)00324-8
- [75] Dias JA, Bonnet B, Weaver BA, Watts J, Kluetzman K, Thomas RM, et al. A negative allosteric modulator demonstrates biased antagonism of the follicle stimulating hormone receptor. *Molecular and Cellular Endocrinology*. 2011;**333**:143-150. DOI: 10.1016/j.mce.2010.12.023
- [76] Dias JA, Campo B, Weaver BA, Watts J, Kluetzman K, Thomas RM, et al. Inhibition of follicle-stimulating hormone-induced preovulatory follicles in rats treated with a nonsteroidal negative allosteric modulator of follicle-stimulating hormone receptor. *Biology of Reproduction*. 2014;**90**:19. DOI: 10.1095/biolreprod.113.109397
- [77] Ayoub MA, Yvinec R, Jégot G, Dias JA, Poli SM, Poupon A, et al. Profiling of FSHR negative allosteric modulators on LH/CGR reveals biased antagonism with implications in steroidogenesis. *Molecular and Cellular Endocrinology*. 2016;**436**:10-22. DOI: 10.1016/j.mce.2016.07.013
- [78] Riccetti L, De Pascali F, Gilioli L, Santi D, Brigante G, Simoni M, et al. Genetics of gonadotropins and their receptors as markers of ovarian reserve and response in controlled ovarian stimulation. *Best practice & research. Clinical Obstetrics and Gynaecology*. 2017;**44**:15-25. DOI: 10.1016/j.bpobgyn.2017.04.002
- [79] Chen C, Xu X, Kong L, Li P, Zhou F, Zhao S, et al. Novel homozygous nonsense mutations in LHCGR lead to empty follicle syndrome and 46, XY disorder of sex development. *Human Reproduction*. 2018;**33**(7):1364-1369. DOI: 10.1093/humrep/dey215
- [80] Binder H, Strick R, Zaherdoust O, Dittrich R, Hamori M, Beckmann MW, et al. Assessment of FSHR variants and antimüllerian hormone in infertility patients with a reduced ovarian response to gonadotropin stimulation. *Fertility and Sterility*. 2012;**97**(5):1169-75.e1. DOI: 10.1016/j.fertnstert.2012.02.012
- [81] Lisi F, Caserta D, Montanino M, Berlinghieri V, Bielli W, Carfagna P, et al. Recombinant luteinizing hormone priming in multiple follicular stimulation for in-vitro fertilization in downregulated patients. *Gynecological Endocrinology*. 2012;**28**(9):674-677. DOI: 10.3109/09513590.2011.652716
- [82] Rahman A, Francomano D, Sagnella F, Lisi F, Manna C. The effect on clinical results of adding recombinant LH in late phase of ovarian stimulation of patients with repeated implantation failure: A pilot study. *European Review for Medical and Pharmacological Sciences*. 2017;**21**(23):5485-5490. DOI: 10.26355/eurrev_201712_13939