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Original Article

Sex-dependent endocrine and cellular effects of the GnRH antagonist degarelix in rabbits and cell models

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Abstract: Background: Degarelix is a long-acting gonadotropin-releasing hormone (GnRH) antagonist that suppresses gonadotropin and sex steroid secretion via competitive blockade of the GnRH receptor (GnRHR). Although its systemic endocrine effects have been clearly identified, its direct effects on non-pituitary-derived cells, as well as the roles of sex and context-dependent pharmacological properties, remain largely unexplored. Methods: Degarelix was profiled in vitro (HEK293T, CHO-K1 cells) and in vivo (male and female New Zealand rabbits). Cell viability was measured using a cell counting kit-8 (CCK-8) assay. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2, in females), and testosterone (T, in males) were quantified by enzyme-linked immunosorbent assay (ELISA). Transcript levels were assessed by quantitative polymerase chain reaction (qPCR), and GnRHR protein abundance and localization were evaluated in hypothalamic-pituitary-gonadal (HPG) tissues using Western blot and immunohistochemistry (IHC). Results: In vitro, degarelix exerted direct, time-dependent, and concentration-dependent effects on the viability of non-pituitary-derived cells ($P < 0.05$), with differential responses observed between HEK293T and CHO-K1 cell lines. In vivo, degarelix induced a biphasic, sex-dependent endocrine response, characterized by a transient elevation (days 1-7) followed by sustained suppression (days 10-28) of gonadotropins and sex steroids, without affecting body weight ($P > 0.05$). qPCR revealed tissue- and sex-specific transcriptional changes, including upregulation of pituitary follicle-stimulating hormone beta subunit (*FSHβ*) mRNA in male rabbits and downregulation of hypothalamic *GnRHR* mRNA in female rabbits. Notably, Western blot and IHC analyses indicated that these mRNA alterations were not accompanied by significant changes in GnRHR protein abundance or localization. Conclusion: This study systematically demonstrates that degarelix exhibits concentration-dependent, sexually dimorphic, and tissue-specific effects in the regulation of reproductive endocrine functions, as well as direct actions on non-pituitary cells. Furthermore, its direct regulation of non-pituitary cells does not depend on changes in GnRHR protein abundance. These findings provide insight into the mechanisms underlying the antagonistic effects of GnRH and lay a theoretical foundation for the personalized application of degarelix in both experimental and clinical settings.

Keywords: GnRH antagonist, degarelix, reproductive endocrinology, cell viability, sex difference

Introduction

The gonadotropin-releasing hormone receptor (GnRHR), a class A G protein-coupled receptor (GPCR), functions as a core regulatory component of the hypothalamic-pituitary-gonadal (HPG) axis due to its specific expression in pituitary gonadotropes [1, 2]. By precisely governing the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), this

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receptor plays a pivotal role in maintaining reproductive endocrine homeostasis, making it a critical therapeutic target for related disorders [3-6]. The long-acting GnRH antagonist degarelix competitively blocks pituitary GnRHR [7-9]. This action rapidly and potently suppresses gonadotropin secretion and subsequent sex hormone levels while effectively circumventing the "flare phenomenon" associated with GnRH agonists. Consequently, degarelix has been established as a first-line clinical agent for hormone-dependent diseases such as prostate cancer [10, 11].

Although the systemic endocrine suppression and clinical safety profile of Degarelix are well established [12], several important questions remain. First, the direct effects on non-target cells and the underlying mechanisms remain unclear. Current research predominantly focuses on its indirect systemic actions via the HPG axis, particularly in specific hormone-related cancer models such as prostate LNCaP or breast cancer cells [13, 14]. In contrast, the functional expression of GnRHR in various extrapituitary tissues - including commonly used non-cancerous cell models such as HEK293T and CHO-K1 - and its potential to mediate direct cellular effects have been relatively neglected. Whether such effects are GnRHR-dependent also remains ambiguous. Second, the sex-dependent dimorphism of its efficacy has not yet been defined. Given the well-recognized sexual dimorphism of the HPG axis, males exhibit relatively stable LH and FSH secretion, whereas females display cyclical fluctuations due to differential GnRHR feedback sensitivity [15]. Notably, the sex-dependent endocrine effects of Degarelix in New Zealand rabbits remain insufficiently characterized. Third, the feedback regulation of the receptor itself is unknown. Although Degarelix blocks GnRHR signaling, its influence on GnRHR transcription and translation, as well as the tissue- and sex-specific regulation of downstream gonadotropin genes (follicle-stimulating hormone beta subunit, *FSHβ*; luteinizing hormone beta subunit, *LHβ*), has not been comprehensively examined [4, 16, 17].

To address these knowledge gaps, we employed an integrated *in vitro* and *in vivo* approach to test the hypothesis that degarelix modulates cell viability and reproductive endocrine function through GnRHR-mediated mechanisms in a concentration-, time-, sex-, and tissue-dependent manner. Using HEK293T and CHO-K1 cells, we assessed cell viability (CCK-8 assay), gene expression (*GnRHR*, *FSHβ*, and *LHβ* by qPCR), and protein expression (GnRHR by Western blotting) to elucidate its direct cellular effects and underlying molecular basis. In male and female New Zealand rabbits, we measured serum hormone levels (FSH, LH, E_2, and T) and analyzed gene expression (qPCR for *GnRHR*, *FSHβ*, *ESR1*, and *AR*), as well as protein expression and localization (Western blotting and immunohistochemistry for GnRHR) in hypothalamic-pituitary-gonadal (HPG) axis tissues. This approach was designed to delineate the sex- and tissue-specificity of its endocrine regulatory effects and to investigate its feedback modulatory actions on GnRHR itself.

This study aims to elucidate the molecular mechanisms underlying degarelix action, clarify its sex- and cell-specific effects, and provide a theoretical basis for optimizing its clinical use in hormone-dependent diseases such as prostate and breast cancer.

Materials and methods

Cell culture and treatments

To investigate whether degarelix exerts direct cellular effects independent of the classical pituitary HPG axis, non-endocrine human embryonic kidney (HEK293T) cells and Chinese hamster ovary (CHO-K1) cells were selected as model systems in this study. Furthermore, all experiments were conducted under natural low receptor conditions, and no genetic interventions targeting GnRHR were introduced. HEK293T and CHO-K1 cells were obtained from Ruibite Biotechnology Co., Ltd. (China). HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), and CHO-K1 cells were cultured in F-12 medium, each supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin-amphotericin B solution. All cells were maintained at 37°C in a humidified incubator containing 5% CO_2.

Degarelix (purity 99.25%; HY-100415, MedChemExpress, Shanghai, China) was dissolved in dimethyl sulfoxide (Dimethyl Sulfoxide, DMSO; HY-Y0475, MedChemExpress, Shanghai, China) to obtain a 5 mM stock solution.

Working concentrations (0-5 μM) were freshly prepared by diluting the stock solution with the appropriate culture medium, ensuring that the final DMSO concentration did not exceed 0.1%, which was confirmed to be non-cytotoxic in preliminary experiments. Cells were treated with degarelix at various concentrations for 24-96 h.

Animal models

Adult male and female New Zealand White rabbits (n = 4 per group; 6 months old; weight range, 2.5-3.5 kg) were obtained from the Animal Center of Jiangsu University. Notably, the estrous cycles of female rabbits were not synchronized in this study to avoid confounding effects from surgical or hormonal interventions; natural cycle variation was therefore acknowledged as an intrinsic variable of the experimental model. Animals were housed under standardized conditions (22 ± 2°C, 50 ± 10% relative humidity, 12 h light/dark cycle) with free access to food and water. All experimental procedures were conducted in accordance with institutional guidelines for animal care and use and were approved by the Animal Ethics Committee of Jiangsu University (IACUC Protocol No. JSU-GnRH-2025).

Collection of blood and tissue samples

New Zealand White rabbits were randomly assigned to either a control group or a degarelix treatment group. The treatment group received a subcutaneous injection of degarelix (200 μg/kg, dissolved in 1 ml of phosphate-buffered saline [PBS]; BL302A, Biosharp, Anhui, China), whereas the control group received an equivalent volume of PBS.

Approximately 2-3 mL of blood was collected from the marginal ear vein of rabbits at 1 day before and at 1, 3, 5, 7, 10, 14, and 28 days after administration. Immediately after collection, blood samples were centrifuged at 3,000 rpm for 20 min to separate serum, which was then stored at -80°C for subsequent hormone analysis. Body weight was measured and recorded concurrently at each blood collection time point. On day 30 after administration, all experimental rabbits were euthanized by excessive carbon dioxide asphyxiation according to institutional animal ethics guidelines and immediately subjected to systematic dissection to collect tissue samples from the hypothalamus, pituitary gland, ovary, uterus, testis, and epididymis. All tissue samples were rinsed with normal saline to remove residual blood. For immunohistochemical (IHC) analysis, testicular tissue samples were fixed in Bouin's solution, while pituitary and ovarian tissue samples were fixed in 4% paraformaldehyde. The remaining tissue samples were stored at -80°C for subsequent quantitative polymerase chain reaction (qPCR) analysis.

Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; CT0001-B, SparkJade, Shandong, China) according to the manufacturer's instructions. Briefly, one day in advance, HEK293T and CHO-K1 cells were seeded into 96-well plates at a density of 5×10^3 cells per well. Cells were cultured at 37°C with 5% CO_2 overnight to allow the cells to adhere to the plate. The cells were treated with various concentrations of degarelix (0-2 μM). At 24, 48, 72, or 96 h after treatment, the culture medium was replaced with fresh medium containing 10% (v/v) CCK-8 solution, and cells were incubated for an additional 1 h at 37°C with 5% CO_2. Absorbance was measured at 450 nm in each reaction well using a microplate reader. Cell viability was expressed as a percentage of the untreated control group and calculated using the following formula:

$$\text{Cell viability (\%)} = \frac{(OD_{450,test} - OD_{450,blank})}{(OD_{450,control} - OD_{450,blank})} \times 100$$

Enzyme-linked immunosorbent assay (ELISA)

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and estradiol (E_2) were quantified using double-antibody, one-step sandwich ELISA kits (AiFang Biological, Hunan, China), according to the manufacturer's instructions. The following kits were used: FSH (AF04196-A), LH (AF04053-A), estradiol (E_2, AF04198-A), and testosterone (T, AF04190-A).

Upon completion of the assay procedure, the optical density (OD) of each well was measured at 450 nm using a microplate reader. Hormone concentrations were calculated from

Table 1. Primer sequence for real-time quantitative PCR

Cell/Tissue	Genes	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')
HEK293T	GAPDH	GTCAAGGCTGAGAACGGGAA	AAATGAGCCCCAGCCTTCT
	GnRHR	CACCTTCTGCCTCTTCA	CTTCAGCCGTGCTCTTGGTA
	FSHβ	GAGCTGACCAACATCACCATTGC	TAGCAGTAGCCAGCACACCAAG
	LHβ	TCTCCTTCCCTGTGGCTCTCAG	CAGGTCAAGGGGTGGTCTTTGG
CHO-K1	GAPDH	GGTTGTCTCCTGCGACTTCA	CCTCTGTTGCTGTAGCCAAC
	GnRHR	CTGGGCAAGCGGAAGTTTTC	TGAAGAGGCAGCTGAAGGTG
	FSHβ	ACCAACATCACCATCGCAGT	GCAATCCTGCGCACCAAG
	LHβ	ATCACCTTCACCACCAGCATCTG	TGCGAAGTGTAGCTCACGGTATG
New Zealand rabbits	GAPDH	GCAAAGTGGATGTTGTCGCC	TGATGACCAGCTTCCCGTTC
	GnRHR	CTCAGCCATCAACAACAGCATCC	AACGGTCACTCGGATCTTTCCAG
	FSHβ	GTACGAGACAGTGAGGGTGC	GTTGCCGCAGTGACATTCAG
	ESR1	GTACGACCCTACCAGACCCT	AGGATCTCTAGCCAGGCACA
	AR	TGGCTCCCTATGGCTACACT	AGGGTCCCATCTCGCTTTTG

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GnRHR, gonadotropin-releasing hormone receptor; FSHβ, follicle-stimulating hormone β-subunit; LHβ, luteinizing hormone β-subunit; ESR1, estrogen receptor 1; AR, androgen receptor.

standard curves generated using kit-provided calibrators.

RNA isolation and quantitative PCR (qPCR)

Total RNA was extracted from HEK293T cells, CHO-K1 cells, and rabbit tissues (hypothalamus, pituitary, testis, epididymis, ovary, and uterus) using TRIzol reagent (CT0001-B, SparkJade, Shandong, China) according to the manufacturer's instructions. RNA concentration and purity were determined spectrophotometrically, and samples with an OD_{260}/OD_{280} ratio between 1.8 and 2.2 were used for subsequent analyses.

First-strand complementary DNA (cDNA) was synthesized using PrimeScript RT Master Mix (Perfect Real Time; CT0001-B, SparkJade, Shandong, China). Quantitative PCR was performed using UltraSYBR Mixture (R433-01, CWBio, Jiangsu, China) on a Bio-Rad CFX96 Real-Time PCR Detection System.

Primer sequences for GnRHR, follicle-stimulating hormone beta subunit (FSHβ), luteinizing hormone beta subunit (LHβ), estrogen receptor 1 (ESR1), androgen receptor (AR), and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed using Primer-BLAST and validated for amplification efficiency. Relative gene expression levels were calculated using the 2^-ΔΔCt method. Primer sequences used in this study are listed in **Table 1**.

Protein extraction and western blotting

HEK293T cells, CHO-K1 cells, and rabbit tissues were lysed in protein lysis buffer containing 1 × RIPA buffer and 1 mM phenylmethanesulfonyl fluoride (PMSF). After centrifugation at 4°C and 13,000 rpm for 15-30 min, the supernatant was collected. 5 × sodium dodecyl sulfate (SDS) sample loading buffer (Sangon Biotech, China) was added at a 4:1 ratio, and the mixture was boiled at 100°C for 5 min to denature proteins. Denatured proteins were separated by 12% sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) (E304-1, Vazyme, Nanjing, China) and transferred onto a polyvinylidene difluoride (PVDF) membrane (IPFL00010, Millipore, Shanghai, China). The membrane was blocked with 5% skim milk at room temperature for 2 h, washed with Tris-buffered saline containing Tween 20 (TBST), and then incubated with the primary antibody against GnRHR (1:2,000 dilution, 19950-1-AP, Proteintech, Wuhan, China) at 4°C overnight. After washing with TBST, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000 dilution, SA000001-2, Proteintech, Wuhan, China) at room temperature for 2 h. Following TBST washes, ultrasensitive enhanced chemiluminescence (ECL) reagent (ED0025-B/C, SparkJade, Shandong, China) was applied for signal detection. Protein bands were visualized by chemiluminescence and quantified using ImageJ software, with GAPDH (1:5,000 dilution, 60004-1-

Ig, Proteintech, Wuhan, China) used as the internal reference.

Immunohistochemistry (IHC)

Tissue samples from New Zealand rabbits were fixed in 4% paraformaldehyde or Bouin's solution (for testes), embedded in paraffin, and sectioned at a thickness of 5 μm. Sections were deparaffinized, rehydrated through a graded ethanol series, and subjected to antigen retrieval. Endogenous peroxidase activity was quenched, and nonspecific binding was blocked using 3% bovine serum albumin (BSA).

Sections were incubated overnight at 4°C with an anti-GnRHR primary antibody (1:200 dilution; 19950-1-AP, Proteintech, Wuhan, China), followed by incubation with a biotinylated secondary antibody and visualization using a diaminobenzidine (DAB) substrate. Nuclei were counterstained with hematoxylin.

Microscopic examination and image acquisition were performed under a light microscope. Hematoxylin stained the cell nuclei blue, whereas DAB-positive immunoreactivity appeared brown to yellow-brown. Staining intensity was semi-quantitatively evaluated using the H-score method:

H-score = $\sum$(Intensity score × Percentage of positive cells)

Where the intensity score was defined as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA, USA). Data are expressed as the mean ± standard error of the mean (SEM). Comparisons between two groups were performed using the Student's *t*-test, whereas comparisons among multiple groups were analyzed using one-way or two-way analysis of variance (ANOVA), followed by Tukey's post hoc test where appropriate. A P value < 0.05 was considered statistically significant.

Results

Differences in viability responses of HEK293T and CHO-K1 cells to degarelix

To evaluate the dose- and time-dependent effects of degarelix on mammalian cell viability, HEK293T and CHO-K1 cells were treated with various concentrations of degarelix (0-2 μM) for 24-96 h, and cell viability was assessed using the CCK-8 assay.

In HEK293T cells, compared with the 0 μM control group, significant reductions in cell viability were observed at 0.2 μM and 2.0 μM after 24 h of treatment (P < 0.01 and P < 0.001, respectively; **Figure 1A**). No significant changes in cell viability were detected at 48 h or 72 h of treatment (all P > 0.05; **Figure 1B**, **1C**). However, treatment with 2.0 μM degarelix resulted in a significant increase in cell viability after 96 h (P < 0.0001; **Figure 1D**), suggesting a potential stimulatory effect following prolonged exposure to a high concentration. In CHO-K1 cells, compared with the 0 μM control group, significant reductions in cell viability were observed at 0.5-2.0 μM degarelix after 24 h of treatment (P < 0.01 to P < 0.0001; **Figure 1E**). At 48 h, treatment with 0.05 μM degarelix resulted in a significant decrease in cell viability (P < 0.001), whereas treatment with 1.0 μM and 2.0 μM degarelix led to significant increases (P < 0.05 to P < 0.001; **Figure 1F**). At 72 h, a significant reduction in cell viability was observed at 1.0 μM degarelix (P < 0.05; **Figure 1G**). At 96 h, cell viability was significantly decreased at 0.2 μM and 0.5 μM degarelix (P < 0.05 to P < 0.001; **Figure 1H**).

Collectively, these findings indicate that degarelix exerts cell line-specific, time-dependent, and dose-dependent effects on cell viability, with significant modulation observed only under specific concentration-exposure time combinations.

Regulatory effects of degarelix on the reproductive endocrine system and body weight in New Zealand rabbits

To evaluate the effects of degarelix on the reproductive endocrine axis, serum concentrations of FSH, LH, estradiol (E_2; females), and testosterone (T; males) were dynamically monitored, and body weight was recorded to assess potential effects on growth and metabolism.

In female rabbits, no significant differences in serum FSH, LH, or E_2 levels were observed between the degarelix and control groups one day before administration (-1 d; P > 0.05; **Figure 2A-C**). During days 1-7 post-administration, levels of all three hormones in the degarelix group

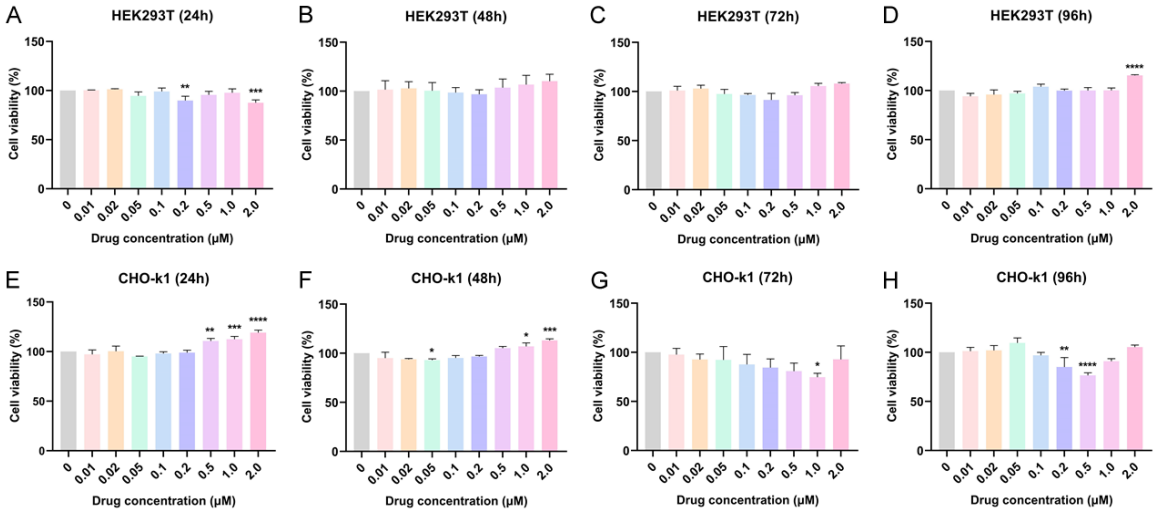

Figure 1. Effects of degarelix on cell viability in HEK293T and CHO-K1 cells. Cell viability was assessed using the CCK-8 assay following treatment with degarelix (0-2 μM) for 24, 48, 72, and 96 h. A-D. Viability of HEK293T cells treated for 24, 48, 72, and 96 h, respectively. E-H. Viability of CHO-K1 cells treated for 24, 48, 72, and 96 h, respectively. Results are presented as a percentage of the control group (mean ± SEM, n = 3). All experiments were independently performed three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

were approximately 1.5-2.0-fold higher than those in the control group ($P < 0.01$ to $P < 0.0001$; **Figure 2A-C**). From days 10-28, hormone levels in the degarelix group decreased to approximately 0.5-0.7-fold of control levels ($P < 0.01$ to $P < 0.0001$; **Figure 2A-C**). These results indicate that degarelix exerts a biphasic, time-dependent regulatory effect on FSH, LH, and E_2 secretion in female rabbits, characterized by an initial stimulatory phase followed by a sustained suppressive phase.

In male rabbits, on the day before dosing (day -1), there was no significant difference in serum testosterone levels between the degarelix and control groups ($P > 0.05$; **Figure 2D-F**), whereas serum FSH and LH levels were significantly higher in the degarelix group ($P < 0.01$ to $P < 0.0001$; **Figure 2D-F**), suggesting potential pre-administration sensitivity. At days 1-7 after administration, FSH, LH, and testosterone levels were significantly higher in the degarelix group than in the control group ($P < 0.01$ to $P < 0.0001$; **Figure 2D-F**). From days 10-28, levels of FSH, LH, and testosterone in the degarelix group were significantly lower than those in the control group ($P < 0.01$ to $P < 0.0001$; **Figure 2D-F**), consistent with a biphasic regulatory pattern similar to that observed in female rabbits.

It is worth noting that no artificial synchronization of estrous cycles was performed in female rabbits in this study, indicating that degarelix may act across different physiological stages of the reproductive cycle. Regarding body weight, both control and degarelix-treated rabbits exhibited a gradual increase over the 28-day observation period, with no significant differences detected at any time point ($P > 0.05$; **Figure 2G**, **2H**). These findings indicate that degarelix does not significantly affect body weight or growth-related metabolic processes in New Zealand rabbits.

Concentration- and time-dependent regulation of gonadotropin and GnRH receptor mRNA expression by degarelix in HEK293T and CHO-K1 cells

To examine the direct regulatory effects of degarelix on gonadotropin-related gene expression, the relative mRNA levels of GnRHR, FSHβ, and LHβ were quantified in HEK293T and CHO-K1 cells after exposure to degarelix (0-5 μM) for 24-96 h.

In HEK293T cells, after 24 h of treatment, FSHβ mRNA expression was significantly upregulated at 0.05 μM, 2 μM, and 5 μM ($P < 0.001$ to $P < 0.0001$; **Figure 3A**), while LHβ mRNA was upregulated at 0.005 μM, 0.02 μM, 0.2 μM,

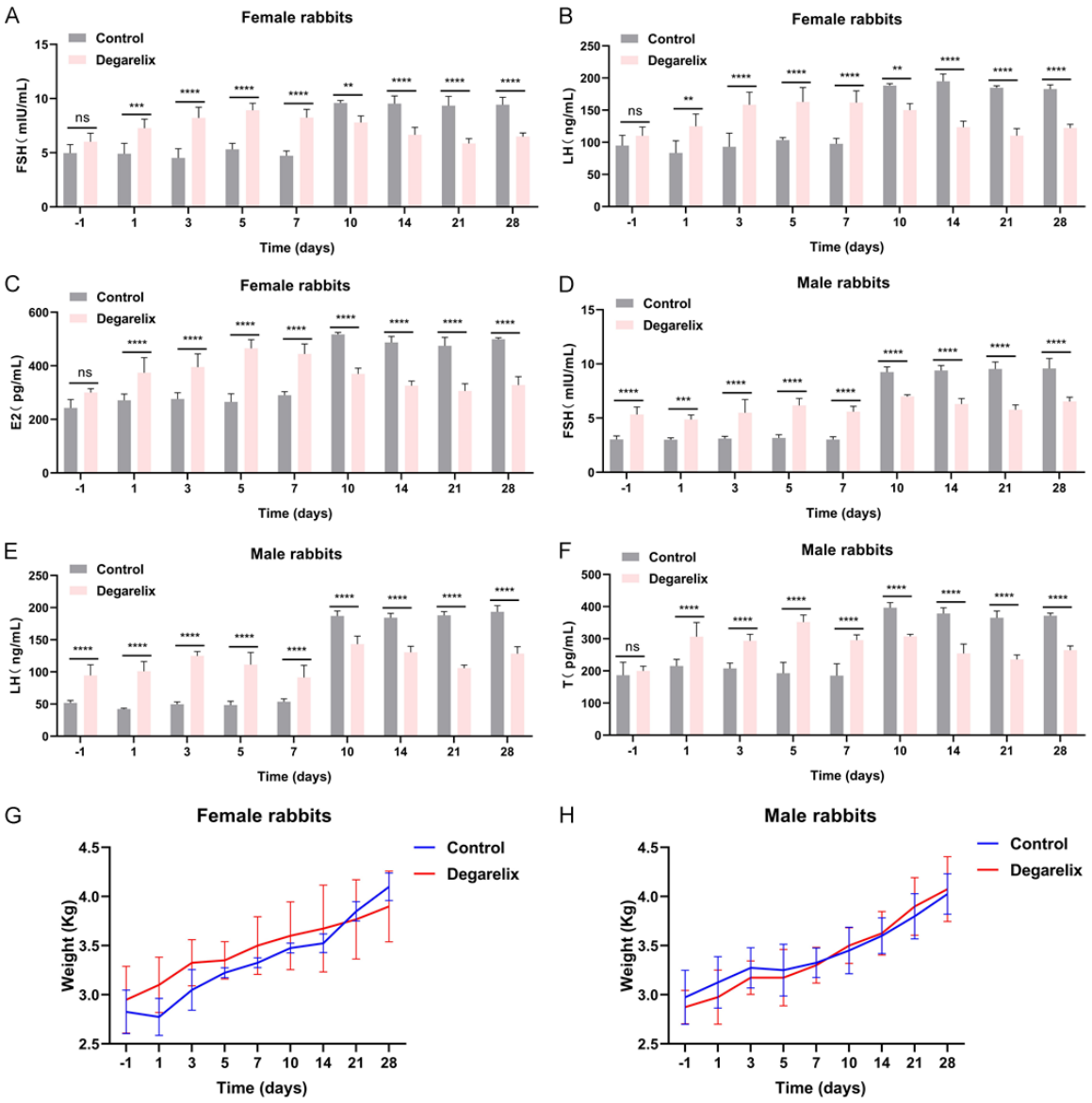

Figure 2. Temporal changes in serum FSH, LH, E_2, and T levels in New Zealand rabbits following degarelix administration. Serum concentrations of FSH, LH, estradiol (E_2), and testosterone (T) were measured by ELISA in male and female rabbits at days -1, 1, 3, 5, 7, 10, 14, and 28 following degarelix treatment. A-C. Time-course changes in serum FSH, LH, and E_2 levels in female rabbits. D-F. Time-course changes in FSH, LH, and T levels in male rabbits. G. Body weight changes of female rabbits. H. Body weight changes of male rabbits. Data are presented as mean ± SEM. All experiments were independently repeated three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

and 5 μM ($P < 0.001$ to $P < 0.0001$; **Figure 3E**). After 48 h, FSHβ mRNA levels were significantly decreased at 0.005-2 μM ($P < 0.05$ to $P < 0.001$), except for an increase observed at 0.01 μM ($P < 0.0001$; **Figure 3B**). At this time point, LHβ expression was significantly elevated only at 5 μM ($P < 0.0001$; **Figure 3F**). Following 72 h and 96 h of exposure, treatment with 5 μM degarelix consistently upregulated both FSHβ and LHβ expression ($P < 0.01$ to $P < 0.0001$; **Figure 3C**, **3D** and **3G**, **3H**). These findings indicate that prolonged exposure to higher concentrations of degarelix exerts a sustained stimulatory effect on gonadotropin subunit gene expression in HEK293T cells.

In CHO-K1 cells, after 24 h of treatment, FSHβ expression was significantly upregulated at 0.005 μM, 0.02 μM, 0.05 μM, 1 μM, and 2 μM ($P < 0.01$ to $P < 0.0001$; **Figure 3I**), and LHβ

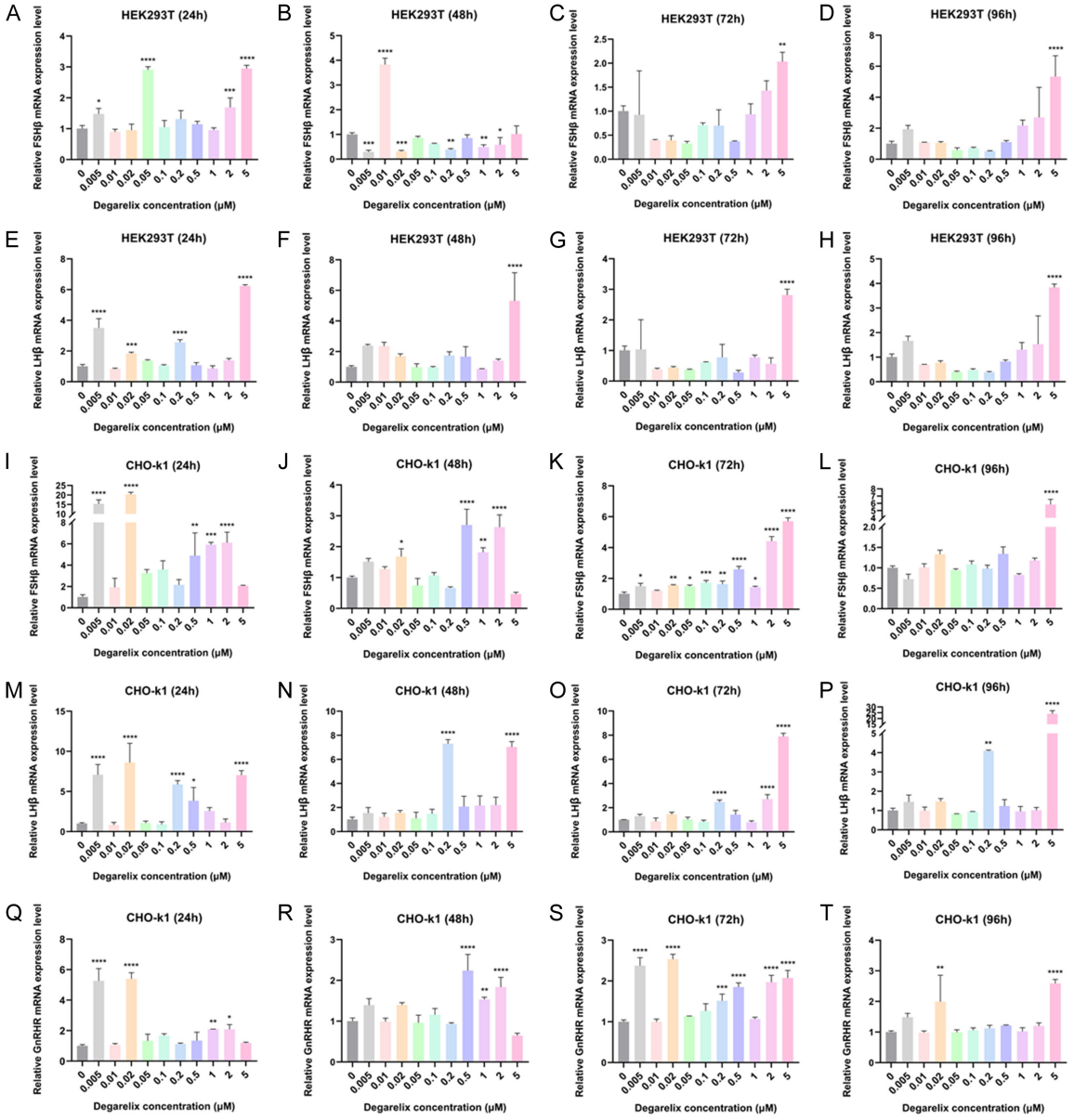

Figure 3. Effects of degarelix on target gene mRNA expression in HEK293T and CHO-K1 cells. Relative mRNA expression levels of *GnRHR*, *FSHβ*, and *LHβ* were quantified by RT-qPCR in HEK293T and CHO-K1 cells treated with varying concentrations of degarelix for 24, 48, 72, and 96 h. *GAPDH* was used as the internal control. A-D. Relative expression of *FSHβ* mRNA in HEK293T cells. E-H. Relative expression of *LHβ* mRNA in HEK293T cells. I-L. Relative expression of *GnRHR* mRNA in CHO-K1 cells. M-P. Relative expression of *FSHβ* mRNA in CHO-K1 cells. Q-T. Relative expression of *LHβ* mRNA in CHO-K1 cells. Data are presented as mean ± SEM. All experiments were independently performed three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

expression was elevated at 0.005 μM, 0.02 μM, 0.2 μM, and 5 μM ($P < 0.05$ to $P < 0.0001$; **Figure 3M**). After 48 h, FSHβ mRNA remained significantly upregulated at 0.02 μM, 0.5 μM, 1 μM, and 2 μM ($P < 0.001$ to $P < 0.0001$; **Figure 3J**), whereas LHβ was upregulated at 0.02 μM and 5 μM ($P < 0.0001$; **Figure 3N**). After 72 h, all concentrations except 0.01 μM significantly increased FSHβ expression ($P < 0.05$ to $P < 0.0001$; **Figure 3K**), and LHβ expression was upregulated at 0.02 μM, 2 μM, and 5 μM ($P < 0.0001$; **Figure 3O**). At 96 h, FSHβ expression remained elevated at 5 μM ($P < 0.0001$; **Figure 3L**), and LHβ was significantly increased at 0.2 μM and 5 μM ($P < 0.01$ to $P < 0.0001$; **Figure 3P**).

Due to the low basal expression of GnRHR in HEK293T cells (Ct ≥ 35), subsequent analysis focused on CHO-K1 cells (**Figure 3Q-T**). After 24 h of treatment, GnRHR mRNA expression was significantly upregulated at 0.005 μM, 0.02 μM, 1 μM, and 2 μM ($P < 0.05$ to $P < 0.0001$; **Figure 3Q**). After 48 h, upregulation was observed at 0.05 μM, 1 μM, and 2 μM ($P < 0.01$ to $P < 0.0001$; **Figure 3R**). After 72 h, GnRHR expression increased at 0.005 μM, 0.02 μM, 0.2 μM, 0.5 μM, 2 μM, and 5 μM ($P < 0.001$ to $P < 0.0001$; **Figure 3S**). At 96 h, significant upregulation persisted at 0.02 μM and 5 μM ($P < 0.01$ to $P < 0.0001$; **Figure 3T**).

Collectively, these findings demonstrate that degarelix regulates FSHβ, LHβ, and GnRHR mRNA expression in a concentration- and time-dependent manner, with CHO-K1 cells exhibiting greater responsiveness and sensitivity than HEK293T cells.

Effects of degarelix on mRNA expression of GnRHR and gonadotropin-related genes in rabbit reproductive tissues

To evaluate tissue- and sex-specific regulatory effects of degarelix, the relative mRNA expression of GnRHR, FSHβ, estrogen receptor 1 (ESR1, in females), and androgen receptor (AR, in males) was quantified in key reproductive tissues of New Zealand rabbits.

In female rabbits, hypothalamic GnRHR mRNA expression was significantly decreased in the degarelix-treated group (0.6-fold of control, $P < 0.05$; **Figure 4A**). No significant changes were observed in GnRHR, FSHβ, or ESR1 mRNA expression in the pituitary, ovary, or uterus ($P > 0.05$; **Figure 4B-J**). In male rabbits, pituitary FSHβ mRNA levels were significantly elevated following degarelix treatment (1.4-fold of control, $P < 0.05$; **Figure 4N**). However, no significant alterations were detected in GnRHR, FSHβ, or AR mRNA expression in the hypothalamus, testis, or epididymis ($P > 0.05$; **Figure 4K-M**, **4O-T**).

Collectively, these results indicate that degarelix modulates gene expression within the reproductive endocrine system in a tissue-specific and sex-dependent manner, primarily affecting hypothalamic GnRHR expression in females and pituitary FSHβ expression in males.

Regulation of GnRHR protein expression by degarelix in HEK293T and CHO-K1 cells: concentration- and time-dependent analysis

To examine whether degarelix influences GnRHR protein expression, Western blotting was performed on HEK293T and CHO-K1 cells treated with degarelix (0-5 μM) for 24-96 h, with GAPDH used as the internal loading control. In both cell lines, the intensity of GnRHR protein bands did not exhibit a clear concentration-dependent pattern at any of the examined time points (**Figure 5A**, **5F**). Quantitative densitometric analysis further confirmed that relative GnRHR protein expression levels did not differ significantly between any degarelix-treated group and the corresponding control ($P > 0.05$ for all comparisons; **Figure 5B-E**, **5G-J**). These findings indicate that within the tested concentration range (0.005-5 μM) and exposure duration (24-96 h), degarelix does not significantly alter GnRHR protein expression in either HEK293T or CHO-K1 cells.

No significant effects of degarelix on GnRHR protein expression or distribution in rabbit reproductive tissues

Western blotting and immunohistochemistry (IHC) were employed to evaluate GnRHR protein expression and localization in reproductive tissues of male and female rabbits following degarelix treatment.

Western blot analysis revealed no significant differences in relative GnRHR protein expression between degarelix-treated and control groups in the hypothalamus, testis, or epididymis of male rabbits ($P > 0.05$ for all comparisons; **Figure 6A-D**), or in the hypothalamus, ovary, and uterus of female rabbits ($P > 0.05$ for all comparisons; **Figure 6E-H**). Similarly, IHC analysis showed no significant changes in GnRHR immunoreactivity, as reflected by comparable H-scores in the pituitary and ovary of female rabbits ($P > 0.05$; **Figure 6I-K**) and in the pituitary and testis of male rabbits ($P > 0.05$; **Figure 6L-N**). Hematoxylin counterstaining confirmed consistent nuclear morphology across groups, and DAB-positive staining (brown to yellow-brown) revealed no evident alterations in GnRHR localization or staining intensity. Collectively, these findings indicate that degarelix does not significantly influence

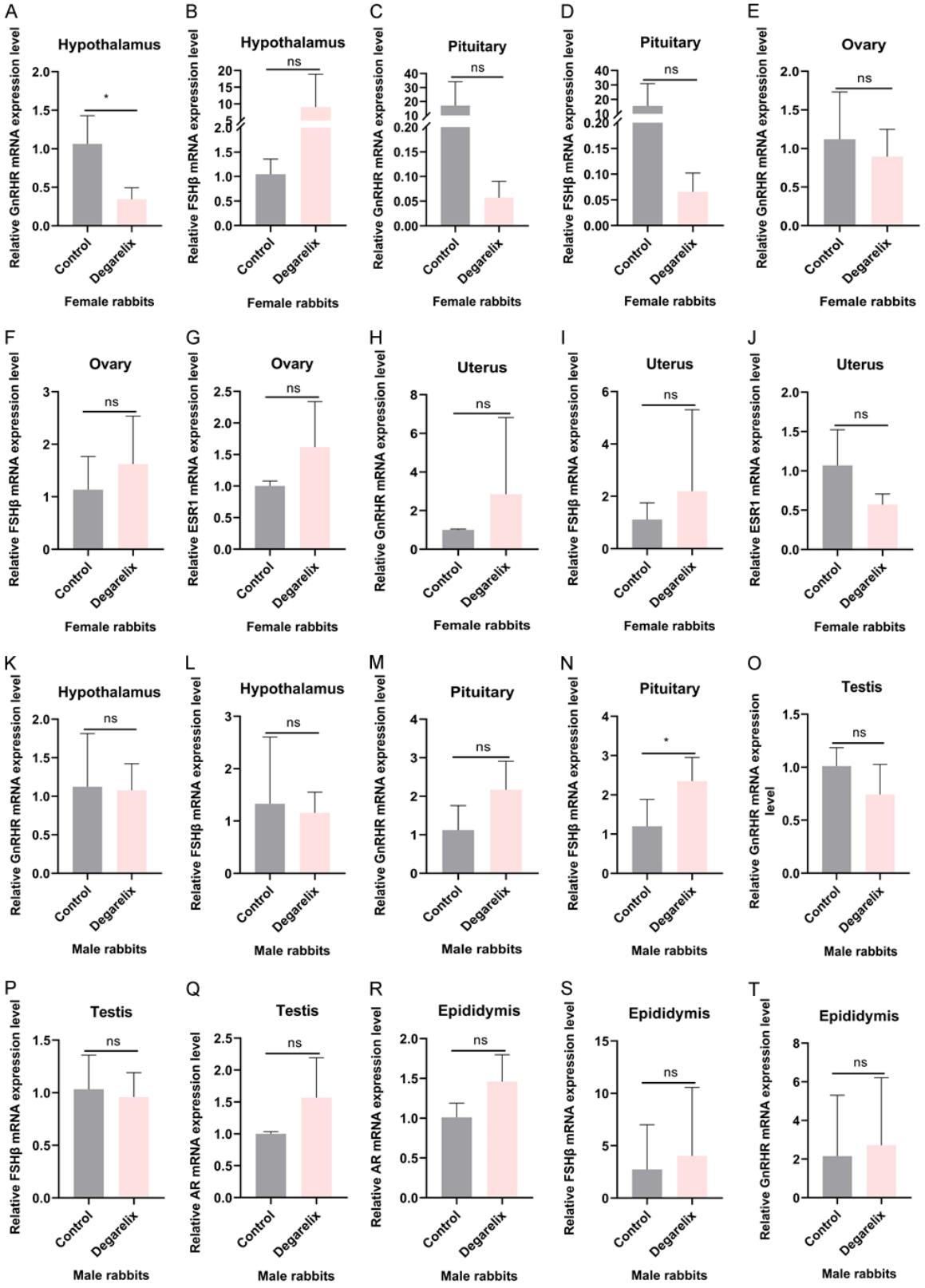

Figure 4. Effects of degarelix on mRNA expression of target genes in reproductive tissues of New Zealand rabbits. Relative mRNA expression levels of *GnRHR*, *FSHβ*, *ESR1*, and *AR* were determined by RT-qPCR in rabbit reproductive tissues (hypothalamus, pituitary, testis, epididymis, ovary, and uterus) following degarelix treatment, with *GAPDH* used as the internal control. A, B. Relative expression of *GnRHR* and *FSHβ* mRNA in the hypothalamus of female rabbits. C, D. Relative expression of *GnRHR* and *FSHβ* mRNA in the pituitary of female rabbits. E-G. Relative

expression of *GnRHR*, *FSHβ*, and *ESR1* mRNA in the ovary of female rabbits. H-J. Relative expression of *GnRHR*, *FSHβ*, and *ESR1* mRNA in the uterus of female rabbits. K, L. Relative expression of *GnRHR* and *FSHβ* mRNA in the hypothalamus of male rabbits. M, N. Relative expression of *GnRHR* and *FSHβ* mRNA in the pituitary of male rabbits. O-Q. Relative expression of *GnRHR*, *FSHβ*, and *AR* mRNA in the testis of male rabbits. R-T. Relative expression of *GnRHR*, *FSHβ*, and *AR* mRNA in the epididymis of male rabbits. Data are presented as mean ± SEM. All experiments were independently performed three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

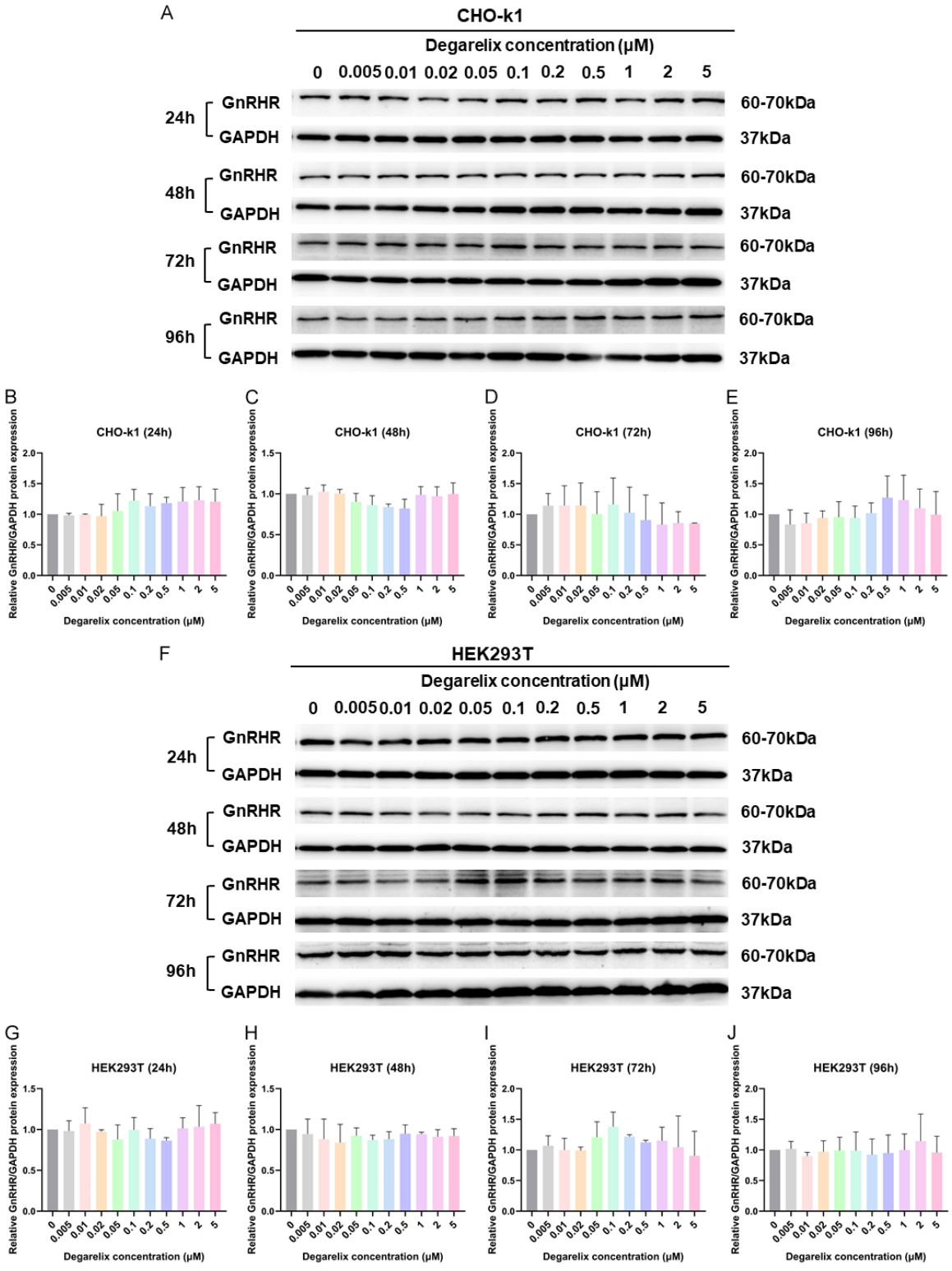

Figure 5. Effects of degarelix on GnRHR protein expression in HEK293T and CHO-K1 cells. GnRHR protein levels were analyzed by Western blotting in HEK293T and CHO-K1 cells treated with varying concentrations of degarelix for 24, 48, 72, and 96 h. GAPDH was used as the internal loading control. A. Representative Western blot showing GnRHR protein expression in CHO-K1 cells treated with different concentrations of degarelix. B-E. Quantitative analysis of GnRHR protein expression in CHO-K1 cells at 24, 48, 72, and 96 h, respectively. F. Representative Western blot showing GnRHR protein expression in HEK293T cells treated with different concentrations of degarelix. G-J. Quantitative analysis of GnRHR protein expression in HEK293T cells at 24, 48, 72, and 96 h, respectively. Data are presented as mean ± SEM. All experiments were independently performed three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

GnRHR protein expression or spatial distribution in rabbit reproductive tissues.

Discussion

Degarelix, as a mature GnRHR antagonist, has been widely used in the treatment of endocrine diseases. This study systematically analyzed the complex effects of the GnRH antagonist degarelix on non-pituitary-derived cells and in whole-animal models using an integrative approach. The observed effects exhibited significant concentration-, time-, sex-, and tissue-dependent characteristics and some of the cellular effects are independent of the abundance of GnRH receptors, suggesting that there may be non-classical signaling pathways.

Experimental model rationale

Our research established a complementary in vitro model employing two internationally recognized standard cell lines, HEK293T and CHO-K1. Both cell lines have characteristic genetic background, high transfection efficiency and good experimental repeatability, and are widely regarded as ideal systems for studying GPCR-mediated signal transduction and drug action mechanisms [18-20]. Specifically, CHO-K1 cells, as a classic model for GPCR research with a complete intracellular signaling network, provide a reliable platform for verifying the pharmacological effects of degarelix via the GnRHR-dependent pathway [21]. In contrast, the endogenous GnRHR expression in HEK293T cells is extremely low, which provides a suitable system for exploring potential non-receptor-dependent mechanisms [22]. Therefore, this dichotomous model enables the distinction between classical receptor-mediated effects and non-classical direct cellular effects of degarelix.

Mechanistic insights into degarelix action

Degarelix is a well-characterized competitive GnRHR antagonist. Its core mechanism involves inhibition of FSH, LH, and downstream sex hormones secretion by blocking GnRHR in the pituitary gland, thereby effectively avoiding the "hormone flare" associated with GnRH agonists [10]. The present study demonstrates that, in addition to its central endocrine actions, degarelix may also directly regulate non-pituitary-derived cells in a cell type-specific, concentration-dependent, and time-dependent manner.

In HEK293T cells, long-term exposure to high concentrations of degarelix unexpectedly enhanced cell viability, suggesting that it may mediate this effect by activating adaptive response mechanisms such as epigenetic regulation [23, 24], although the specific molecular mechanism remains to be further elucidated. In contrast, CHO-K1 cells exhibited a more complex and dynamic response pattern, characterized by fluctuating effects across different concentrations and exposure times, indicating that the action of degarelix was significantly selective and environment-dependent. This result is consistent with the report of Kuchiara V et al. [25], which pointed out that degarelix inhibited proliferation in most prostate-derived cell lines (e.g., WPE1-NA22, WPMY-1, BPH-1, VCaP, LNCaP, C4-2BMDVR, and CWR22Rv1), but showed no effect in PC-3 cells. These differential effects further highlight the critical role of cellular heterogeneity in determining drug responsiveness, including multiple factors such as receptor expression levels, configuration of intracellular signaling networks, and metabolic characteristics [26-29].

Endocrine regulation and sexual dimorphism

Our *in vivo* results demonstrated a time-dependent, bidirectional effect of degarelix on reproductive hormone regulation in rabbits. During the early stage after administration (days 1-7), serum levels of FSH, LH, E_2, and T were significantly increased, whereas during long-term treatment (days 10-28), these levels shifted to sustained inhibition. This "brief early increase"

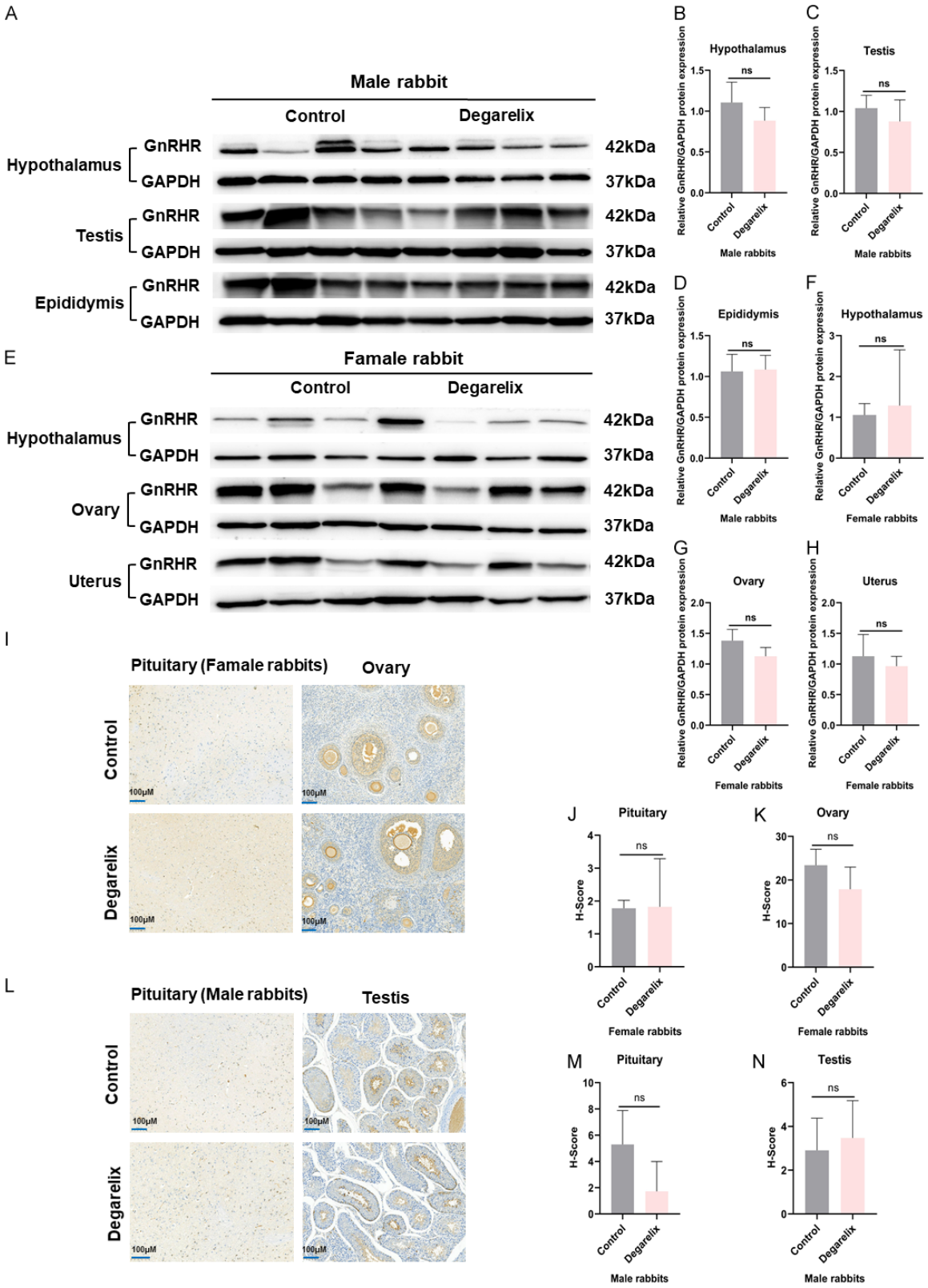

Figure 6. Effects of degarelix on GnRHR protein expression and localization in reproductive tissues of New Zealand rabbits. GnRHR protein expression was analyzed by Western blotting, and GnRHR localization was assessed by immunohistochemistry (IHC) using DAB staining with hematoxylin nuclear counterstaining in rabbit reproductive tissues. (A) Representative Western blots showing GnRHR protein expression in male rabbit tissues (pituitary, testis,

and epididymis). (B-D) Quantitative analysis of GnRHR protein expression corresponding to (A). (E) Representative Western blots showing GnRHR protein expression in female rabbit tissues (pituitary, ovary, and uterus). (F-H) Quantitative analysis of GnRHR protein expression corresponding to (E). (I) Representative IHC staining of GnRHR in the pituitary and ovary of female rabbits (control and degarelix-treated groups). (J, K) Quantitative analysis (H-score) of GnRHR IHC staining in female rabbit tissues. (L) Representative IHC staining of GnRHR in the pituitary and testis of male rabbits (control and degarelix-treated groups). (M, N) Quantitative analysis (H-score) of GnRHR IHC staining in male rabbit tissues. Scale bar = 100 μm. Data are presented as mean ± SEM (n = 3-4). All experiments were independently repeated three times. Statistical significance is indicated as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$.

kinetic characteristic is somewhat reminiscent of the transient stimulatory phase triggered by GnRH agonists [7]. However, previous clinical studies have not reported similar hormone "surges" or "micro-surges" in patients receiving degarelix [10, 30-33]. This discrepancy may be related to species-specific differences in receptor-ligand affinity or pharmacokinetics [34]. Furthermore, New Zealand rabbits are induced ovulators and lack a regular estrous cycle. In this study, estrous cycles were not synchronized, implying that female rabbits may have been at different physiological stages with spontaneous time-dependent fluctuations in gonadotropin and sex steroid levels. This physiological characteristic may partly explain the gradual increase in hormone levels observed in the control group [35, 36].

Moreover, the observed sexual dimorphism in endocrine responses is consistent with findings reported by Chang *et al.* [15]. This sex difference may be attributed to the inherent dimorphism of HPG axis function [37]. In males, testosterone secretion is relatively stable and primarily regulated by negative feedback, whereas in females it is influenced by cyclical estrogen fluctuations and both negative and positive feedback mechanisms. This fundamental difference may render males more sensitive to changes in receptor occupancy, resulting in a more rapid endocrine response. Although previous studies have suggested that weight gain may be a potential side effect of degarelix [30], no significant trend of weight increase was observed in the present study, indicating that metabolic effects may exhibit species- or dose-dependent differences.

Transcriptomic regulation and noncanonical mechanisms

Degarelix markedly influenced FSHβ, LHβ, and GnRHR mRNA expression in CHO-K1 cells in a distinct concentration- and time-dependent manner, revealing complex temporal patterns of gene regulation [38]. As a reversible GnRHR antagonist, degarelix is conventionally understood to suppress downstream signaling through competitive receptor blockade [10, 30, 31]. However, the observed upregulation of gonadotropin subunit genes expands the current understanding of antagonist pharmacodynamics. Beyond simple receptor blockade, degarelix may induce biased signaling, feedback activation, or metabolic reprogramming that leads to secondary transcriptional modulation [39-42]. The divergent transcriptional responses of FSHβ and LHβ suggest differences in promoter sensitivity, transcription factor recruitment, or regulatory architecture [27, 43]. Furthermore, the non-monotonic dose-response patterns observed for FSHβ and LHβ transcription are consistent with threshold- and feedback-dependent GnRH/GnRHR signaling. In this context, intermediate antagonist concentrations may be insufficient to elicit sustained transcriptional activation, whereas low or high concentrations may engage distinct signaling pathways or adaptive regulatory mechanisms [27]. Notably, the expression level of GnRHR in HEK293T cells was extremely low, suggesting that part of the effect of degarelix on the viability of these cells may be independent of classical receptor-mediated signaling, further illustrating the critical role of the cellular microenvironment in explaining its pharmacodynamic response [44, 45].

Protein-level regulation and post-translational modifications

Although changes in GnRHR mRNA expression were observed, degarelix did not significantly alter the abundance or localization of GnRHR protein, suggesting that it has limited effects on the direct biosynthesis or degradation processes of the receptor [1, 46]. As a GPCR, GnRHR function is primarily regulated by conformational dynamics, coupling efficiency with

G proteins, and rapid membrane trafficking processes, including receptor phosphorylation, internalization, and recycling [3, 6, 41, 47-50]. The discrepancy between mRNA and protein levels observed in this study is consistent with the fact that the regulation of GPCR function is more dependent on post-transcriptional mechanisms and membrane dynamics than on protein content alone. In contrast to GnRH agonists (such as triptorelin), which typically upregulate receptor expression, the antagonist degarelix likely operates through distinct molecular mechanisms [51]. Furthermore, Western blot analysis revealed that the apparent molecular weight of the GnRHR band was higher than predicted, suggesting the presence of post-translational modifications, such as glycosylation [52, 53]. These modifications could impede antibody recognition, thereby reducing detection sensitivity and complicating accurate protein quantification.

Tissue-specific and sex-dependent gene regulation

Degarelix exerted tissue-specific and sex-dependent effects on gene expression within the rabbit reproductive axis. Specifically, it downregulated hypothalamic GnRHR mRNA in females while upregulating pituitary FSHβ expression in males. Suppression of hypothalamic GnRHR, a key regulator of the HPG axis, may reflect a negative feedback regulatory mechanism triggered by the blockade of endogenous GnRH signaling [16, 35]. Sex-specific responses may result from heterogeneity of pituitary GnRHR isoforms, differences in downstream signaling cascades, or differences in hormonal microenvironments [27, 35]. In addition, the unique reproductive physiological characteristics of rabbits as ovulatory animals may further exacerbate the complexity of these effects [36]. Collectively, these findings emphasize that GnRH antagonist activity is shaped by a sexually dimorphic neuroendocrine environment. This context-dependent regulation carries important implications for expanding the therapeutic use of degarelix beyond prostate cancer to female reproductive disorders, such as breast cancer, ovarian cancer, polycystic ovary syndrome, and assisted reproductive technologies (ART) [13, 54, 55].

Limitations and future directions

This study has several limitations, including a limited sample size, unsynchronized estrous cycles in female rabbits, a lack of early (0.5-24 h) endocrine monitoring, and insufficient analysis of apoptosis, cell-cycle progression, and gene-editing effects. The direct anti-tumor activity of degarelix was also not assessed in standard tumor cell lines, restricting the evaluation of its clinical potential. These factors may affect the generalizability and mechanistic depth of the findings. Future work should establish GnRHR-overexpression models to confirm receptor-dependent mechanisms, integrate Pharmacokinetics-Pharmacodynamics (PK-PD) modeling to link drug exposure with endocrine responses, and further clarify the roles of MAPK/PKC signaling and the basis of sex-specific differences in degarelix effects.

Conclusion

In conclusion, this study demonstrates that degarelix exerts sex-specific regulatory effects on the reproductive endocrine axis in rabbits and induces context-dependent alterations in cellular viability *in vitro*, without significantly affecting GnRHR protein expression. These findings indicate that the direct actions of degarelix can occur independently of receptor abundance. Collectively, these findings highlight the mechanistic complexity of GnRH antagonist activity, expanding current understanding of their multimodal pharmacological effects. This improved mechanistic insight provides a theoretical basis for dose optimization and individualized monitoring in the clinical management of hormone-dependent diseases, such as prostate cancer and breast cancer. Future studies integrating tumor cell models with gene-editing technologies may further elucidate degarelix's mechanisms of action under conditions of low GnRHR expression or receptor mutation, thereby generating critical data to support the development of precision therapeutic strategies.

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Disclosure of conflict of interest

None.

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References

[1] Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K and Maudsley SR. Gonadotropin-releasing hormone receptors. Endocr Rev 2004; 25: 235-275.

[2] Kakar SS and Jennes L. Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor mRNAs in various non-reproductive human tissues. Cancer Lett 1995; 98: 57-62.

[3] Zhang M, Chen T, Lu X, Lan X, Chen Z and Lu S. G protein-coupled receptors (GPCRs): advances in structures, mechanisms, and drug discovery. Signal Transduct Target Ther 2024; 9: 88.

[4] Tzoupis H, Nteli A, Androutsou ME and Tselios T. Gonadotropin-releasing hormone and GnRH receptor: structure, function and drug development. Curr Med Chem 2020; 27: 6136-6158.

[5] Lee G, Cheung AP, Ge B, Zhu M, Giolma B, Li B, Wong E, Li Y, Wang Y, Chen Z and Gu J. CA215 and GnRH receptor as targets for cancer therapy. Cancer Immunol Immunother 2012; 61: 1805-1817.

[6] Lorente JS, Sokolov AV, Ferguson G, Schioth HB, Hauser AS and Gloriam DE. GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov 2025; 24: 458-479.

[7] Patel S, Saxena B, Mehta P and Niazi SK. GnRH peptide antagonist: comparative analysis of chemistry and formulation with implications for clinical safety and efficacy. Pharmaceuticals (Basel) 2024; 18: 36.

[8] Zhao J, Chen J, Sun G, Shen P and Zeng H. Luteinizing hormone-releasing hormone receptor agonists and antagonists in prostate cancer: effects on long-term survival and combined therapy with next-generation hormonal agents. Cancer Biol Med 2024; 21: 1012-1032.

[9] Oduwole OO, Poliandri A, Okolo A, Rawson P, Doroszko M, Chrusciel M, Rahman NA, Serrano De Almeida G, Bevan CL, Koechling W and Huhtaniemi IT. Follicle-stimulating hormone promotes growth of human prostate cancer cell line-derived tumor xenografts. FASEB J 2021; 35: e21464.

[10] Carter NJ and Keam SJ. Degarelix: a review of its use in patients with prostate cancer. Drugs 2014; 74: 699-712.

[11] Limonta P, Montagnani Marelli M, Mai S, Motta M, Martini L and Moretti RM. GnRH receptors in cancer: from cell biology to novel targeted therapeutic strategies. Endocr Rev 2012; 33: 784-811.

[12] Lopes RD, Higano CS, Slovin SF, Nelson AJ, Bigelow R, Sørensen PS, Melloni C, Goodman SG, Evans CP, Nilsson J, Bhatt DL, Clarke NW, Olesen TK, Doyle-Olsen BT, Kristensen H, Arney L, Roe MT, Alexander JH, Mol-Arts M, Mansor-Lefebvre S, Zubovskiy K, Blemings A, Dugi K, Bloomfield G, Kontos C, DeVore A, Jordan D, Kolls B, Matthews R, Mehta R, Povsic TJ, Morse M, Mahaffey KW, Halabi S, Leong D, Klotz L, Fleshner N, Jansz G, Giddens J, Egerdie R, Chin J, Zadra J, Casey R, Simard J, Niazi T, Martin A, Babjuk M, Hajek J, Klecka J, Kubes J, Schraml J, Jakesova J, Vanasek J, Melichar B, Seikkula H, Abdiche MS, Colombel M, Debourdeau P, Robert G, Villers A, Ploussard G, Pradere B, Bruyere F, Descotes J, Ouzaid I, Winter A, Hanitzsch H, Sperling H, Eckert R, Hammerer P, Stagge E, Seseke F, Szymula S, Bamias A, Thanos A, Hatzimouratidis K, Mamoulakis C, Kalofonos H, Oszukowska E, Madziarska K, Fijuth J, Obarzanowski M, Alekseev B, Atduev V, Pushkar D, Veliev E, Zyryanov A, Petrov S, Kopyltsov E, Kozlov V, Macko L, Dubravicky J, Polak R, Mir O, Vargovcak M, Mincik I, Kliment J, Goncalves F, Mikulas J, Sokol R, Korcek M, Marko J, Kovacik V, Milichovsky I, Dubinsky P, Lazarus J, Dixit S, Green E, Srinivasan R, Mazhar D, Ng Y, Sarwar N, Herman C, Snoy F, Given R, Suh R, Lipsitz D, Bailen J, Gervasi L, Acosta I, Belkoff L, Wu N, Frankel J, Karsh L, Poole B, Lieber D, Engel J, Bidair M, Rosenberg S, Sieber P, Perzin A, Kalota S, Singh A, Henderson R, Wayne J, Mirza M, D Anna R, Wolk F, Padron O, Bylow K, Rubenstein J, Gartrell B, Schwartz M, Patel K, Maniam A, Keane T, Goodman M, Bane C, Chung M, Savage S, Uchio E, Nguyen S, Aronson W, Khanna R and Barnswell C. Cardiovascular Safety of Degarelix Versus Leuprolide in Patients With Prostate Cancer: The Primary Results of the PRONOUNCE Randomized Trial. Circulation. 2021; 144: 1295-1307.

[13] Mondaca JM, Muñoz JMF, Barraza GA, Vanderhoeven F, Redondo AL, Flamini MI and Sanchez AM. Therapeutic potential of GNRHR analogs and SRC/FAK inhibitors to counteract tumor growth and metastasis in breast cancer. Biochim Biophys Acta Mol Basis Dis 2025; 1871: 167826.

[14] Sakai M, Martinez-Arguelles DB, Patterson NH, Chaurand P and Papadopoulos V. In search of the molecular mechanisms mediating the inhibitory effect of the GnRH antagonist degarelix on human prostate cell growth. PLoS One 2015; 10: e120670.
[15] Chang B, Wall EG, Herbison AE and Han SY. Comparative analysis of GnRH pulse generator activity in intact and gonadectomized male and female mice. Endocrinology 2025; 166: bqaf099.
[16] Magee C, Kouri JE, Cherrington BD, Cantlon JD, Murtazina DA, Farmerie TA, Davidsen MH, Nett TM and Clay CM. Evidence for divergent endocrine regulation of the murine and ovine GnRH receptor gene promoters. Front Endocrinol (Lausanne) 2025; 16: 1597028.
[17] Fontana F and Limonta P. Dissecting the hormonal signaling landscape in castration-resistant prostate cancer. Cells 2021; 10: 1133.
[18] Ju MS, Ahn HM, Han SG, Ko S, Na JH, Jo M, Lim CS, Ko BJ, Yu YG, Lee WK, Kim YJ and Jung ST. A human antibody against human endothelin receptor type A that exhibits antitumor potency. Exp Mol Med 2021; 53: 1437-1448.
[19] Zhang C, Tong F, Zhou B, He M, Liu S, Zhou X, Ma Q, Feng T, Du WJ, Yang H, Xu H, Xiao L, Xu ZZ, Zhu C, Wu R, Wang YQ and Han Q. TMC6 functions as a GPCR-like receptor to sense noxious heat via Gαq signaling. Cell Discov 2024; 10: 66.
[20] Küffer A, Lakkaraju AK, Mogha A, Petersen SC, Airich K, Doucerain C, Marpakwar R, Bakirci P, Senatore A, Monnard A, Schiavi C, Nuvolone M, Grosshans B, Hornemann S, Bassilana F, Monk KR and Aguzzi A. The prion protein is an agonistic ligand of the G protein-coupled receptor Adgrg6. Nature 2016; 536: 464-468.
[21] Chen ZG, Yu KL, Zheng HM and Dong KW. Estrogen receptor-mediated repression of gonadotropin-releasing hormone (gnRH) promoter activity in transfected CHO-K1 cells. Mol Cell Endocrinol 1999; 158: 131-142.
[22] Yang S, Zhang L, Khan K, Travers J, Huang R, Jovanovic VM, Veeramachaneni R, Sakamuru S, Tristan CA, Davis EE, Klumpp-Thomas C, Witt KL, Simeonov A, Shaw ND and Xia M. Identification of environmental compounds that may trigger early female puberty by activating human GnRHR and KISS1R. Endocrinology 2024; 165: bqae103.
[23] Sugawara T, Nevedomskaya E, Heller S, Bohme A, Lesche R, von Ahsen O, Grunewald S, Nguyen HM, Corey E, Baumgart SJ, Georgi V, Putter V, Fernandez-Montalvan A, Vasta JD, Robers MB, Politz O, Mumberg D and Haendler B. Dual targeting of the androgen receptor and PI3K/AKT/mTOR pathways in prostate cancer models improves antitumor efficacy and promotes cell apoptosis. Mol Oncol 2024; 18: 726-742.
[24] Xie H, Hoffmann HM, Iyer AK, Brayman MJ, Ngo C, Sunshine MJ and Mellon PL. Chromatin status and transcription factor binding to gonadotropin promoters in gonadotrope cell lines. Reprod Biol Endocrinol 2017; 15: 86.
[25] Cucchiara V, Yang JC, Liu C, Adomat HH, Tomlinson Guns ES, Gleave ME, Gao AC and Evans CP. GnRH antagonists have direct inhibitory effects on castration-resistant prostate cancer via intracrine androgen and AR-V7 expression. Mol Cancer Ther 2019; 18: 1811-1821.
[26] Sakai M, Elhilali M and Papadopoulos V. The GnRH antagonist degarelix directly inhibits benign prostate hyperplasia cell growth. Horm Metab Res 2015; 47: 925-931.
[27] Stamatiades GA and Kaiser UB. Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol Cell Endocrinol 2018; 463: 131-141.
[28] Sonesson A, Koechling W, Stalewski J, Tankó LB and Rasmussen BB. Metabolite profiles of degarelix, a new gonadotropin-releasing hormone receptor antagonist, in rat, dog, and monkey. Drug Metab Dispos 2011; 39: 1895-1903.
[29] Broqua P, Riviere PJ, Conn PM, Rivier JE, Aubert ML and Junien JL. Pharmacological profile of a new, potent, and long-acting gonadotropin-releasing hormone antagonist: degarelix. J Pharmacol Exp Ther 2002; 301: 95-102.
[30] Steinberg M. Degarelix: a gonadotropin-releasing hormone antagonist for the management of prostate cancer. Clin Ther 2009; 31: 2312-2331.
[31] Shore ND. Experience with degarelix in the treatment of prostate cancer. Ther Adv Urol 2013; 5: 11-24.
[32] Rick FG, Block NL and Schally AV. An update on the use of degarelix in the treatment of advanced hormone-dependent prostate cancer. Onco Targets Ther 2013; 6: 391-402.
[33] Liu YF, Fu SQ, Yan YC, Gong BB, Xie WJ, Yang XR, Sun T and Ma M. Progress in clinical research on gonadotropin-releasing hormone receptor antagonists for the treatment of prostate cancer. Drug Des Devel Ther 2021; 15: 639-649.
[34] Thau RB, Limonta P, Schmidt F and Sundaram K. Species differences in the sensitivity to GnRH analogs. J Steroid Biochem 1985; 23: 811-817.
[35] Ramakrishnappa N, Rajamahendran R, Lin YM and Leung PCK. GnRH in non-hypothalamic reproductive tissues. Anim Reprod Sci 2005; 88: 95-113.

[36] Foote RH and Carney EW. The rabbit as a model for reproductive and developmental toxicity studies. Reprod Toxicol 2000; 14: 477-493.
[37] Yael A, Fishman R, Matas D, Doniger T, Vortman Y and Koren L. Fetal endocrine axes mRNA expression levels are related to sex and intrauterine position. Biol Sex Differ 2024; 15: 61.
[38] Yeung CM, An BS, Cheng CK, Chow BK and Leung PC. Expression and transcriptional regulation of the GnRH receptor gene in human neuronal cells. Mol Hum Reprod 2005; 11: 837-842.
[39] Kenakin T. Biased receptor signaling in drug discovery. Pharmacol Rev 2019; 71: 267-315.
[40] Hapgood JP, Sadie H, Van Biljon W and Ronacher K. Regulation of expression of mammalian gonadotrophin-releasing hormone receptor genes. J Neuroendocrinol 2005; 17: 619-638.
[41] Chaudhary PK and Kim S. An insight into GPCR and G-proteins as cancer drivers. Cells 2021; 10: 3288.
[42] Counis R, Laverrière JN, Garrel G, Bleux C, Cohen-Tannoudji J, Lerrant Y, Kottler ML and Magre S. Gonadotropin-releasing hormone and the control of gonadotrope function. Reprod Nutr Dev 2005; 45: 243-254.
[43] Odle AK, MacNicol MC, Childs GV and MacNicol AM. Post-transcriptional regulation of Gnrhr: a checkpoint for metabolic control of female reproduction. Int J Mol Sci 2021; 22: 3312.
[44] Sanno N, Jin L, Qian X, Osamura RY, Scheithauer BW, Kovacs K and Lloyd RV. Gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor messenger ribonucleic acids expression in nontumorous and neoplastic pituitaries. J Clin Endocrinol Metab 1997; 82: 1974-1982.
[45] Ishaq M, Schang AL, Magre S, Laverrière JN, Guillou A, Coudouel N, Wargnier R, Cohen-Tannoudji J and Counis R. Rat Gnrhr promoter directs species-specific gene expression in the pituitary and testes of transgenic mice. J Mol Endocrinol 2013; 50: 411-426.
[46] Koechling W, Hjortkjaer R and Tankó LB. Degarelix, a novel GnRH antagonist, causes minimal histamine release compared with cetrorelix, abarelix and ganirelix in an ex vivo model of human skin samples. Br J Clin Pharmacol 2010; 70: 580-587.
[47] Li S, Chen J, Chen X, Yu J, Guo Y, Li M and Pu X. Therapeutic and prognostic potential of GPCRs in prostate cancer from multi-omics landscape. Front Pharmacol 2022; 13: 997664.
[48] Addis P, Bali U, Baron F, Campbell A, Harborne S, Jagger L, Milne G, Pearce M, Rosethorne EM, Satchell R, Swift D, Young B and Unitt JF. Key aspects of modern GPCR drug discovery. SLAS Discov 2024; 29: 1-22.
[49] Wang W, Qiao Y and Li Z. New insights into modes of GPCR activation. Trends Pharmacol Sci 2018; 39: 367-386.
[50] Zhang Q, Jiang B, Du Z and Chasin LA. A doubly auxotrophic CHO-K1 cell line for the production of recombinant monoclonal antibodies. Biotechnol Bioeng 2020; 117: 2401-2409.
[51] Xu Y, Li W, Shi M, Xu X, Guo D, Liu Z, Chen L, Zhong X and Cao W. Systemic treatment with GnRH agonist produces antidepressant-like effects in LPS induced depression male mouse model. Neuropharmacology 2023; 233: 109560.
[52] Davidson JS, Flanagan CA, Davies PD, Hapgood J, Myburgh D, Elario R, Millar RP, Forrest-Owen W and McArdle CA. Incorporation of an additional glycosylation site enhances expression of functional human gonadotropin-releasing hormone receptor. Endocrine 1996; 4: 207-212.
[53] Xu X, Nagarajan H, Lewis NE, Pan S, Cai Z, Liu X, Chen W, Xie M, Wang W, Hammond S, Andersen MR, Neff N, Passarelli B, Koh W, Fan HC, Wang J, Gui Y, Lee KH, Betenbaugh MJ, Quake SR, Famili I, Palsson BO and Wang J. The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. Nat Biotechnol 2011; 29: 735-741.
[54] Dellapasqua S, Gray KP, Munzone E, Rubino D, Gianni L, Johansson H, Viale G, Ribi K, Bernhard J, Kammler R, Maibach R, Rabaglio-Poretti M, Ruepp B, Di Leo A, Coates AS, Gelber RD, Regan MM, Goldhirsch A and Colleoni M; International Breast Cancer Study Group. Neoadjuvant degarelix versus triptorelin in premenopausal patients who receive letrozole for locally advanced endocrine-responsive breast cancer: a randomized phase II trial. J Clin Oncol 2019; 37: 386-395.
[55] Papanikolaou EG, Yarali H, Timotheou E, Grynberg M, Zafeiratis O, Tournaye H and Najdecki R. A proof-of-concept clinical trial of a single luteal use of long-acting gonadotropin-releasing hormone antagonist degarelix in controlled ovarian stimulation for in vitro fertilization: long antagonist protocol. Front Endocrinol (Lausanne) 2018; 9: 25.