Cetrorelix Acetate: A Novel Approach to Ovulation Induction in India

Module 1

Cetrorelix Acetate: A Breakthrough in Ovulation Induction

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Infertility and Ovulation Induction

Infertility affects 5–15% of women worldwide, with around half of those affected seeking medical assistance. Assisted reproductive technology (ART) has become an essential approach for couples struggling with infertility. During controlled ovarian stimulation (COS) cycles, one of the primary concerns is premature ovulation, which can occur due to an early luteinizing hormone (LH) surge. Premature ovulation can lead to cycle cancellations or the retrieval of poor-quality oocytes, both of which significantly impact pregnancy rates.

In normal physiology, the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the synthesis and release of follicle-stimulating hormone (FSH) and LH from the anterior pituitary. In ART, pituitary suppression is vital for controlling ovarian stimulation by exogenous FSH while also preventing the mid-cycle LH surge. This is achieved through the administration of GnRH analogues, which are either agonists or antagonists.

GnRH antagonists, which were first introduced to clinical practice in 1999, offer a therapeutic alternative to GnRH agonists. Cetrorelix is one such antagonist, and its primary mechanism involves competing with native GnRH for binding to pituitary cell membrane receptors. This action leads to the rapid and dose-dependent suppression of LH, thereby controlling premature LH surges.

Compared to GnRH agonists, antagonists like cetrorelix have several advantages. They produce immediate and reversible suppression of LH and FSH without causing the initial "flare-up" effect seen with agonists. Therefore, prolonged pretreatment to downregulate the pituitary is not required. Since GnRH antagonists are typically administered only during the risk period for premature LH surge (from day 5 to 7 of stimulation), symptoms related to hypoestrogenism are uncommon. Moreover, using GnRH antagonists results in a shorter total cycle duration and fewer days of exogenous gonadotropin stimulation compared to agonists. This allows subsequent ART cycles to be initiated more quickly.

GnRH antagonists like cetrorelix also offer practical benefits for patients with specific clinical conditions. For example, patients who are at high risk for ovarian hyperstimulation syndrome (OHSS), such as those with polycystic ovary syndrome (PCOS), may benefit from the controlled use of GnRH antagonists, which reduce the number of ovarian follicles compared to long agonist protocols. Early evidence suggests a lower incidence of moderate or severe

OHSS in high-risk patients when a GnRH agonist is used to trigger oocyte maturation instead of human chorionic gonadotropin (hCG).

In addition to OHSS prevention, the effective suppression of LH by GnRH antagonists has shown promise in cycles of ovulation induction (OI) for patients with PCOS, which is characterized by chronic ovulatory dysfunction and hypersecretion of LH. The avoidance of an untimely LH surge is particularly helpful in scheduling intrauterine insemination (IUI) following OI.

A scheme illustrating the medication course based on cycle day during ovarian stimulation with the short antagonist protocol is presented in **Figure 1**. **Table 1** provides a comparison of GnRH antagonists and GnRH agonists.

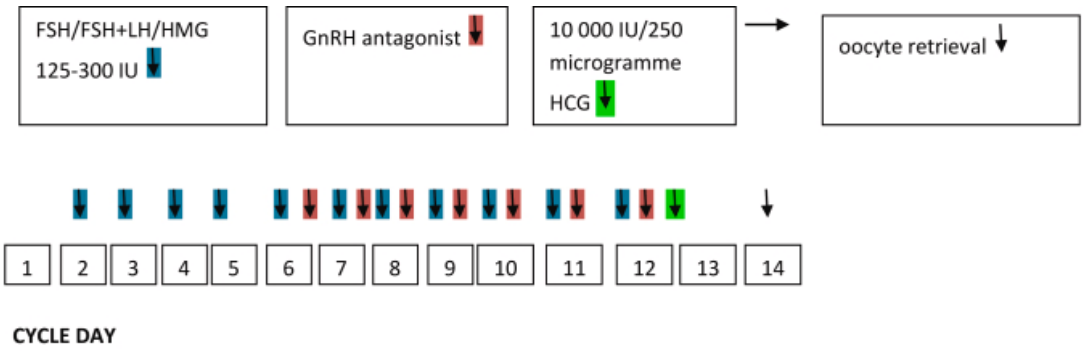
Table 1: Comparison of the GnRH antagonist cetrorelix with GnRH analogs.

Criteria	GnRH antagonist cetrorelix	GnRH agonists
Onset of action	Immediate	Delayed (flare-up effect)
Efficacy	High	High
Route of administration	Subcutaneous injection	Subcutaneous injection
Daily dose	1–2 injections	One injection per day or depot preparation
Indications	Controlled ovarian stimulation, ovarian cancer, prostate cancer, benign prostate hyperplasia, fertility preservation	Controlled ovarian stimulation, endometriosis, fibroids, prostate cancer, fertility preservation
Risk profile	favorable (minimal risk for OHSS)	unfavorable (risk for OHSS)
Risk for local allergic reaction	higher	lower
Mode of action	competitive binding to the GnRH receptors to suppress the GnRH effects	down-regulation of the GnRH receptors and desensitization of the gonadotrophic cells
Reversibility	immediately	after 6 weeks

Clinical benefits	simple stimulation no increase of gonadotrophin dose at GnRH antagonist initiation avoidance of OHSS and ovarian cysts shorter stimulation lower gonadotrophin consumption	more oocytes retrieved potential suppression of endometriosis

GnRH = gonadotropin-releasing hormone; OHSS = ovarian hyperstimulation syndrome.

Figure 1: Short antagonist protocol applied in IVF and ICSI cycles



ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

The Development and Role of Cetrorelix in ART

Cetrorelix, a decapeptide belonging to the GnRH antagonist group, works by inhibiting LH secretion from the anterior pituitary, preventing ovulation, and controlling sex steroid production. Initially used in oncology, cetrorelix has become a vital part of ART, particularly in short antagonist protocols for COS. The drug is administered subcutaneously and is generally well-tolerated, though some patients may experience localized skin reactions such as redness, swelling, or pain.

The need for GnRH antagonists like cetrorelix arose from the challenge of premature LH surges during gonadotropin stimulation. The positive feedback from estradiol on the pituitary gland leads to premature ovulation, reducing oocyte and embryo quality, which in turn lowers pregnancy rates. In response, significant research led to the synthesis of GnRH agonists and, later, antagonists such as cetrorelix.

Cetrorelix was first introduced to the European market for controlled ovarian stimulation in 1999. Shortly thereafter, it became a standard in reproductive medicine, replacing GnRH agonists in many protocols due to its superior safety profile and efficacy. Its ability to prevent OHSS by allowing ovulation to be triggered with a GnRH agonist rather than hCG has been particularly beneficial in reducing the risks associated with gonadotropin stimulation.

Although earlier studies suggested a lower pregnancy rate with the short antagonist protocol, subsequent research demonstrated that this approach is as effective as the long agonist protocol, particularly in terms of live birth rates. In fact, the European Society of Human Reproduction and Embryology now recommends the short antagonist protocol as the standard due to its enhanced safety for patients and its ability to reduce both the duration of stimulation and gonadotropin consumption.

Overall, cetrorelix has become a cornerstone in ART treatments such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). With its excellent safety profile and effective prevention of OHSS, it remains one of the key GnRH antagonists in modern reproductive medicine.

Chemistry of Cetrorelix

Cetrorelix is a basic synthetic peptide with a molecular weight of 1431.06 g/mol (calculated as the anhydrous free base). It acts as a structural analogue of native gonadotropin-releasing hormone (GnRH) but functions as a GnRH antagonist, meaning it blocks the receptor without affecting ovarian steroidogenesis. This synthetic decapeptide differs from native GnRH due to substitutions at amino acid positions 1, 2, 3, 6, and 10. These modifications, particularly the use of D-amino acids, enhance the bioavailability of cetrorelix by reducing its enzymatic degradation in humans.

Cetrorelix is available in sterile, lyophilized powder form for subcutaneous injection, offered in 0.25-mg or 3.0-mg doses. It is reconstituted with sterile water for injection (pH 5–8). The 0.25-mg vial is diluted with 1.0 ml of water (equivalent to 0.26–0.27 mg cetrorelix acetate), while the 3.0-mg vial is diluted with 3.0 ml of water (equivalent to 3.12–3.24 mg cetrorelix acetate).

Pharmacology of Cetrorelix

The native GnRH peptide stimulates the release of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary's gonadotropic cells. Mid-cycle, rising estradiol levels trigger a positive feedback mechanism, leading to the midcycle LH surge that induces ovulation, oocyte meiosis, and luteinization. Cetrorelix works by competitively binding to GnRH receptors on pituitary cells, blocking them and thus inhibiting the secretion of LH and FSH in a dose-dependent manner.

After administration of cetrorelix, LH suppression occurs within approximately 1 hour (for the 3.0-mg dose) or 2 hours (for the 0.25-mg dose). Continuous administration maintains suppression, with the effect on LH being more pronounced than on FSH. Unlike GnRH agonists, cetrorelix does not cause an initial flare-up of gonadotropins due to its immediate antagonist action.

Once cetrorelix treatment is stopped, LH and FSH levels fully recover. This reversible effect has been observed in both animal studies and human trials. By delaying the LH surge, cetrorelix also suppresses ovulation in women, with the degree of suppression being dose-dependent. The effect of a single 3.0-mg dose lasts for up to 4 days, with a maximum suppression of 70% on

day 4. For a 0.25-mg dose, the suppressive effect is maintained with daily administration every 24 hours.

Pharmacodynamics of Cetrorelix

GnRH antagonists, such as cetrorelix, rapidly suppress pituitary gonadotropin release and prevent premature LH surges in both single- and multiple-dose protocols. Phase I trials have demonstrated that a single 0.25-mg dose of cetrorelix for controlled ovarian stimulation (COS) reduces LH levels by 90% within 3 hours, with minimal amplitude pulses observed during suppression. When a 3.0-mg dose of cetrorelix is administered on day 8 of a spontaneous cycle, LH levels significantly drop within 75 minutes, reaching a nadir at 16 hours and reducing by 91% compared to pre-treatment values. The LH levels remain low for at least 96 hours following the single 3.0-mg dose.

In another study where a 0.25-mg dose of cetrorelix was given on day 3 of the cycle, LH was suppressed by 75%, with the lowest levels reached 6 hours post-injection. A prospective observational trial examining the suppressive effect of cetrorelix in five women using a multiple-dose protocol for COS found that LH levels decreased by 73%, with LH reaching its lowest point 429 minutes after injection. Throughout the 32-hour observation period, 16 significant secretory peaks were detected, with a mean interpulse interval of 112 minutes. Cetrorelix significantly suppressed LH pulses for 456 minutes, after which secretion resumed, though with decreased amplitude and pulse mass.

Pharmacokinetics

Cetrorelix is rapidly absorbed following subcutaneous administration, with peak plasma levels (Tmax) reached within 1 to 2 hours. The drug is metabolized by peptidases and has an absolute bioavailability of approximately 85% in healthy female subjects. After a 3.0-mg intravenous dose, the volume of distribution is about 1.16 l/kg, and in vitro studies show that cetrorelix has a plasma protein binding rate of 86%. Cetrorelix concentrations in the follicular fluid and plasma are similar in patients undergoing COS. On the day of oocyte pick-up (OPU) and embryo transfer, plasma concentrations of cetrorelix are typically below or near the lower limit of quantification after subcutaneous doses of 0.25 mg or 3.0 mg. There is no significant impact on hormone levels other than the suppression of the LH surge during COS.

Pharmacokinetic studies show no significant differences between healthy individuals and patients undergoing COS. However, enzyme elevations (e.g., alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase) up to three times the upper limit of normal occurred in 1–2% of patients treated with cetrorelix, excluding those with pre-existing conditions. No pharmacokinetic studies have been conducted in patients with renal or liver impairment, elderly individuals, or children. Cetrorelix is contraindicated in individuals with severe renal impairment and is classified as Pregnancy Category X due to evidence of serious fetal abnormalities.

Metabolism

Studies on cetrorelix metabolism in animals (rats and dogs) have shown that rats excreted 24.3% of cetrorelix via urine and 69.6% through feces, with excretion nearly complete within 48 hours and no enteral reabsorption detected. Dogs reached Tmax within 1.3 hours. In humans, the plasma half-life (t½) following a single 0.25-mg intravenous dose is 12 hours, while subcutaneous administration of the same dose results in a t½ of 30 hours. The difference in half-lives is attributed to absorption processes at the injection site. After a single 3.0-mg subcutaneous dose, the elimination half-life is 63 hours, while it is only 5 hours after a 0.25-mg subcutaneous dose. In a multiple-dose regimen, the t½ is 21 hours.

Linear pharmacokinetics are observed with subcutaneous administration of cetrorelix over 14 days. Following the administration of 10 mg subcutaneously, unchanged cetrorelix was detected in the urine, while small amounts of its metabolites (nona-, hepta-, hexa-, and tetrapeptides) were found in bile samples within 24 hours. Approximately 2–4% of the administered dose was recovered as unchanged cetrorelix in urine, and 5–10% was excreted as cetrorelix and its metabolites in bile. The tetrapeptide (1-4) was the predominant metabolite. Only 7–14% of the administered dose was recovered as unchanged cetrorelix and metabolites within 24 hours, and the remaining portion may have been recovered with longer urine and bile collection periods.

In vitro studies show that cetrorelix is stable against Phase I and II metabolism, and drug-to-drug interactions have not been investigated.

Phase I studies

Based on promising preclinical results that demonstrated efficacy and a favorable safety profile, Cetrorelix progressed to Phase I clinical trials involving human volunteers. Across 15 separate Phase I studies, the effects of single and multiple subcutaneous (s.c.) injections, as well as single intravenous (i.v.) infusions, were assessed. In total, Cetrorelix was administered to 236 healthy participants, including 161 men and 75 women. The doses tested in these studies ranged from 0.25 mg to 20 mg per s.c. injection. The findings from representative studies are summarized below.

Initial Studies on Cetrorelix in Male Volunteers

The first administration of Cetrorelix in humans involved single s.c. doses administered to healthy male volunteers. The study revealed that the extent and duration of testosterone suppression increased with higher doses. A dose of 1 mg of Cetrorelix resulted in a maximum testosterone suppression of 73% eight hours after injection. Higher doses of 2 mg and 5 mg led to testosterone suppression of 80% and 91%, respectively. Testosterone levels began to recover within 48 hours after the 5 mg dose, reaching serum concentrations comparable to those of the placebo group and within the lower normal range. Interestingly, the suppression of follicle-stimulating hormone (FSH) did not reach statistical significance. Pharmacokinetically, linear kinetics were observed, with a calculated plasma half-life of 30 hours following a 5 mg dose.

Cetrorelix Administration in Premenopausal Women

In preparation for its clinical use in in vitro fertilization (IVF) programs, single doses of Cetrorelix were administered to healthy premenopausal women. Doses of 3 mg and 5 mg were given between days 6 and 10 of the menstrual cycle, resulting in immediate decreases in serum luteinizing hormone (LH), FSH, and estradiol levels. A nadir was reached 24 hours post-injection, with reductions of 56% for LH, 29.5% for FSH, and 85% for estradiol compared to baseline. There was no significant difference in suppression between the two doses. Importantly, in all cases, the LH surge was delayed, occurring 6 to 17 days after the Cetrorelix injection. When administered during the late follicular phase—when plasma estradiol concentrations exceeded 150 pg/ml—spontaneous LH surges were similarly postponed.

However, FSH suppression was less pronounced, leading to early discussions about the potential for using reduced stimulation protocols during controlled ovarian stimulation cycles.

Pharmacokinetics and Bioavailability in Male Volunteers

To further investigate the pharmacokinetics, bioavailability, and pharmacodynamic effects of Cetrorelix, a study was conducted in which healthy male volunteers received both i.v. and s.c. administrations of 3 mg doses, with a 21-day washout period between doses. The study showed that testosterone suppression was most pronounced, with mean decreases of 93% after i.v. administration and 95% after s.c. injection. LH levels were reduced by 82% (i.v.) and 80% (s.c.), whereas FSH levels exhibited a less dramatic decrease of 41% and 49%, respectively. This study also provided important safety and tolerability data for Cetrorelix.

Multiple Dosing Regimens in Male Volunteers

Multiple dose studies in men explored the effects of daily administration of Cetrorelix over 7, 8, or 14 days, with doses ranging from 0.25 mg to 10 mg per day. Daily dosing over 8 days showed dose-dependent suppression of gonadotropins and testosterone. Interestingly, only a high dosage of 10 mg/day was able to maintain testosterone at castration levels for the entire treatment period. Lower doses led to a rebound increase in testosterone concentrations between days 2 and 4 of treatment. Based on these findings and insights from animal studies, researchers developed a dosing regimen that combined an initial high dose with subsequent low-dose maintenance. A regimen consisting of 10 mg/day for 5 days followed by 1 mg/day successfully maintained testosterone suppression within the castration range. This treatment also resulted in a marked and reversible reduction in prostate volume by approximately 40% within two weeks.

Studies in Female Volunteers

Cetrorelix was also tested in female volunteers over three consecutive menstrual cycles: pre-treatment, treatment, and post-treatment. Participants received daily doses of 3 mg Cetrorelix for one week, with the first dose administered on day 8 of their cycle. Within 24 hours of the first injection, LH levels were significantly suppressed, and estradiol levels dropped to postmenopausal values. The suppressive effects of Cetrorelix persisted after the last injection, with a mean duration of 13 days for LH suppression, 9.4 days for FSH suppression, and 14.6 days for estradiol suppression. On day 15 of treatment, LH levels had decreased to 16.1% of

baseline values, FSH levels to 63.5%, and estradiol to postmenopausal levels. After treatment cessation, an LH surge followed by post-ovulatory progesterone levels occurred in all women.

Pharmacokinetic Analysis and Dose-Linearity in Women

Pharmacokinetic studies in female volunteers revealed dose-linearity, with daily doses of 0.25 mg, 0.5 mg, and 1 mg administered from cycle days 3 to 16. A single 3 mg dose resulted in a plasma half-life of approximately 8 hours after i.v. administration and 25 hours after s.c. injection. The bioavailability of Cetrorelix was determined to be 92%. These findings provided crucial insights into the pharmacokinetic properties and clinical potential of Cetrorelix, particularly in the context of IVF and other fertility treatments.

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Early LHRH antagonists were associated with histamine release, leading to severe local and systemic side effects such as edema and anaphylactoid reactions. As a precaution, an intradermal skin test was often performed before administering therapeutic doses. In initial studies, intracutaneous tests with 10 mg of Cetrorelix were used to identify patients at risk of severe allergic reactions. However, no systemic adverse effects were observed during these trials. Mild local reactions, such as redness at the injection site, were common but transient.

Further studies involving 25 healthy volunteers of both sexes revealed that repeated intracutaneous tests with 7-10 mg of Cetrorelix did not produce reproducible skin effects. These tests were not predictive of local or systemic side effects during subsequent subcutaneous (s.c.) treatments. The most frequent local reaction after s.c. doses was mild to moderate erythema, which resolved spontaneously within minutes. The size of erythema varied greatly between individuals, and there was no correlation between the frequency of local reactions and the number of consecutive administrations. These reactions occurred independently of dose, sex, or even after placebo administration.

Other side effects were related to the pharmacodynamic action of Cetrorelix, such as reduced testosterone or estradiol levels, resulting in decreased libido and hot flashes. Laboratory parameters showed no significant changes after single doses, although multiple-dose treatments led to time- and dose-dependent increases in high-density lipoprotein (HDL) cholesterol levels. No significant changes were observed in low-density lipoprotein (LDL) cholesterol or triglyceride concentrations.

Phase II Studies in Different Indications

For ovarian stimulation, human menopausal gonadotrophin (HMG) or, more recently, recombinant follicle-stimulating hormone (rFSH) is utilized, followed by ovulation induction using human chorionic gonadotrophin (HCG), which is administered when a sufficient number of mature follicles are present. Assisted reproductive techniques, such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), are subsequently employed to obtain embryos for uterine transfer.

The traditional stimulation procedure with gonadotrophins presents challenges, including unpredictable ovarian responses and the risk of premature LH surges, which can occur due to the positive feedback from rising oestradiol levels in up to 25% of cases. These surges may negatively impact oocyte quality, while increased progesterone levels can adversely affect the endometrium, ultimately reducing pregnancy rates and potentially leading to cycle cancellations.

To mitigate premature LH surges, LHRH agonists have been incorporated into stimulation protocols to suppress endogenous LH, thus reducing the frequency of premature luteinization to approximately 2%. Currently, the "long protocol," which begins agonist treatment at least 14 days prior to stimulation with gonadotrophins, is considered the most effective method. However, this approach has several drawbacks:

1. Extended treatment duration before gonadotrophin suppression allows for the initiation of ovarian stimulation.
2. Prolonged exposure to hormonal medications.
3. Strong suppression of both gonadotrophins and oestradiol can lead to hormone withdrawal symptoms, such as hot flashes, and necessitates higher doses of HMG or FSH for stimulation.
4. Induced down-regulation of receptors and depletion of gonadotrophin storage vesicles require a recovery period for pituitary responsiveness, which may contribute to the need for luteal phase support.

Cetrorelix in Ovarian Stimulation

Recent advancements in treatment modalities have incorporated Cetrorelix, which allows for immediate suppression of gonadotrophins. This advancement helps to avoid the unwanted stimulatory phase created by LHRH agonists and significantly shortens treatment duration by administering Cetrorelix only during the heightened risk for premature LH surges.

Phase II clinical trials involving 294 patients were conducted to evaluate the efficacy and safety of Cetrorelix in controlled ovarian stimulation for assisted reproduction techniques using HMG, as rFSH had not yet been registered in Europe when the study commenced. Two dosing regimens of Cetrorelix were implemented: multiple doses (3, 1, 0.5, 0.25, and 0.1 mg/day) beginning on cycle day 5 or 6 until HCG administration, and a single or dual dose (5, 3, and 2 mg) primarily on stimulation day 7.

In initial studies, patients with primary or secondary tubal sterility received daily subcutaneous injections of Cetrorelix, starting on day 7 of the menstrual cycle. No endogenous LH surge was observed, and following Cetrorelix administration, LH values decreased immediately. Oestradiol concentrations remained stable, indicating continuous follicular development. Oocyte quantity and quality were comparable to results seen with the long protocol using agonists. All patients successfully had oocytes collected and fertilized, achieving a fertilization rate of 61.5%. The requirement for gonadotrophin ampoules was reduced to 27, compared to 35-40 with the long protocol.

Further studies assessed lower doses of Cetrorelix, identifying a daily subcutaneous dose of 0.25 mg as the minimal effective dose to prevent premature LH surges while maintaining good oocyte quality.

To streamline the stimulation protocol, the effectiveness of a single injection was also evaluated. A study with 17 patients assessed the action duration of a single 5 mg dose of Cetrorelix, resulting in no premature LH surges. Subsequent patients received a single dose of 3 mg on day 8 of the menstrual cycle, confirming that this dose was generally effective in preventing premature LH surges.

Overall, the Phase II development of Cetrorelix in controlled ovarian stimulation for assisted reproduction achieved a pregnancy rate of 30% per embryo transfer.

Phase III Trials and Further Investigations

To confirm these findings, a Phase III trial program was initiated, encompassing three multicentric, multinational studies. Multiple doses of 0.25 mg/day of Cetrorelix starting on stimulation day 5 or 6 were tested, with a control group receiving Buserelin nasal spray. Additionally, the efficacy of a single dose of 3 mg Cetrorelix on stimulation day 7 and a single 3.75 mg triptorelin depot in the control group were evaluated. Results are pending publication, but it is anticipated that Cetrorelix will yield comparable outcomes in terms of the number of

follicles, oocytes retrieved, fertilization rates, and pregnancy rates compared to patients treated with LHRH agonists.

The ability of Cetrorelix to prevent premature LH surges opens up various options for inducing final oocyte maturation and ovulation. Due to the preserved responsiveness of the pituitary to LHRH during Cetrorelix treatment, studies have explored administering a single injection of an LHRH agonist or recombinant LH to induce ovulation, potentially benefiting patients at high risk for ovarian hyperstimulation syndrome and those with polycystic ovarian diseas

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