GnRH agonist as novel luteal support: results of a randomized, parallel group, feasibility study using intranasal administration of buserelin*

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BACKGROUND: The study objective was to investigate whether repeated intranasal administration of a GnRH agonist could provide convenient and safe luteal support. METHODS: Twenty-four patients with unexplained infertility were enrolled. All patients were treated with an aromatase inhibitor. When ovulation trigger criteria were met, patients were randomly allocated to either 5000 IU hCG (group A), or 200 μg intranasal buserelin followed by 100 μg every 3 days (group B), 100 μg every 2 days (group C), or 100 μg every day (group D), up to day 14 of the luteal phase. All patients underwent intrauterine insemination. RESULTS: Follicular development was similar in all groups with 1.1 ± 0.3 follicles ≥16 mm, 229.4 ± 95.2 pg/ml estradiol (E2) and 0.8 ± 0.5 ng/ml progesterone (mean ± SD). The luteal phase duration (median; 95% confidence interval) was 15 (14.1, 15.0), 14 (12.5, 15.5), 15 (11.8, 18.2) and 15 (14.4, 15.6) days in groups A, B, C and D respectively. From luteal phase day 7 onwards, progesterone levels tended to be higher in group D compared with A. On day 14 of the luteal phase, progesterone levels were 3.0 (0.8, 5.2), 1.7 (–0.5, 3.9), 3.9 (–0.7, 8.5) and 7.7 (3.4, 11.9) ng/ml in groups A, B, C and D respectively (P = 0.045). No pregnancy was recorded in group A, but there was one biochemical pregnancy in group B, one biochemical and one singleton clinical pregnancy in group C, and two singleton clinical pregnancies in group D. CONCLUSION: Intranasal administration of buserelin could be effective to provide luteal support. This treatment was associated with a good pregnancy rate (5/18, 28%).

Key words: aromatase inhibitor/buserelin/GnRH agonist/intrauterine insemination/luteal support

Introduction

Luteal phase deficiency is a common feature of cycles resulting from stimulation of follicular development. Luteal phase deficiency has been reported in cycles stimulated with hMG/FSH alone, in cycles down-regulated with a GnRH agonist and stimulated with hMG/FSH (Macklon and Fauser, 2000; Pritts and Atwood, 2002), as well as in cycles using a GnRH antagonist in combination with hMG/FSH (Kolibianakis et al., 2003; Beckers et al., 2003).

Luteal phase supplementation or support is therefore common practice in infertility treatment to significantly improve embryo implantation rates, clinical pregnancy rates and delivery rates (for review see Pritts and Atwood, 2002).

Two therapeutic agents are routinely used to supplement the luteal phase: natural progesterone and hCG. In a meta-analysis of prospective randomized trials, the clinical pregnancy odds ratio for luteal support by hCG versus no treatment was found to be 2.72 [confidence interval (CI): 1.56, 4.90; P < 0.05] and the clinical pregnancy odds ratio for i.m. progesterone versus no treatment was found to be 2.38 (CI: 1.36, 4.27; P < 0.05) (Pritts and Atwood, 2002). Vaginal administration of progesterone is probably as effective as i.m. progesterone in multiple daily applications, whereas the efficacy of a single daily administration has been questioned (Propst et al., 2001; Pritts and Atwood, 2002).

The recent introduction of GnRH competitive antagonists as a substitute for GnRH agonists, to prevent mistimed LH surges, has again opened up the possibility of using a GnRH agonist to induce final follicular maturation (Olivennes et al., 1996; Fauser et al., 2002; Kol, 2004). However, with this new treatment paradigm, the luteal phase has also been shown to be deficient and luteal support is necessary (Albano et al., 1999; Beckers et al., 2003; Kolibianakis et al., 2003).

We therefore postulated that the LH-releasing property of a GnRH agonist could be exploited in non-down-regulated cycles, not only to trigger final follicular maturation but also

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as luteal support. This new approach could offer several advantages over current treatments: (i) convenient nasal self-administration, compared to hCG injections or multiple daily vaginal administrations of progesterone; (ii) use of purely synthetic polypeptide with no biological fluid residues; (iii) the opportunity for early diagnosis of pregnancy.

Because GnRH agonists may induce desensitization, the risk of failure to support the luteal phase was deemed significant and, for a first study, it was decided to test the concept in patients undergoing treatment less invasive than assisted reproduction techniques, i.e. an intrauterine insemination (IUI) cycle. The logic behind it was that, although luteal support may not be necessary in this model, if the GnRH agonist were to induce desensitization and luteolysis, it would not be worth testing it further in assisted reproduction patients.

We aimed to assess whether GnRH agonist administered intranasally can trigger ovulation and support the luteal phase without inducing desensitization in patients undergoing mild ovarian stimulation and IUI.

Materials and methods

Study design

This was a single centre, randomized, open, parallel group, pilot study aimed at testing the feasibility of using an intranasally administered GnRH agonist (buserelin) to trigger ovulation and support the luteal phase of patients undergoing IUI after stimulation of follicular development with exemestane. Three doses of buserelin were to be tested and information on efficacy and safety was to be collected. The study protocol and the informed consent form were approved by the institution’s ethics committee.

Patients and treatment groups

Twenty-four patients with unexplained infertility indicating mild stimulation of follicular development associated with IUI treatment were recruited into the study. Diagnosis of unexplained infertility was based on ovulation assessment, laparoscopic evaluation of the pelvis and tubal patency, as well as male partner sperm assessment. After signing the informed consent form, patients were to undergo mild ovarian stimulation using an aromatase inhibitor (Exemestane; Pfizer, Belgium) at a dose of 25 mg/day (oral tablets) from day 2 to day 6 of a spontaneous cycle. Exemestane was selected because, in contrast to letrozole, it is not teratogenic (Exemestane Product Label (2002)). When a patient met the criteria to trigger ovulation (at least one follicle ≥18 mm), she was randomized to one of four treatment arms: (i) hCG 5000 IU (subcutaneous administration) (Pregnyl; Organon, The Netherlands) (control, n = 6, group A); (ii) 200 µg buserelin (Suprefact; Aventis, Belgium) intranasally followed by 100 µg buserelin every 3 days intranasally (n = 6, group B); (iii) 200 µg buserelin intranasally followed by 100 µg buserelin every 2 days intranasally (n = 6, group C); or (iv) 200 µg buserelin intranasally followed by 100 µg buserelin every day intranasally (n = 6, group D). The first dose of buserelin to trigger ovulation was selected based on a previous dose-finding study (Buckett et al., 1998). The dose to be administered during the luteal phase was selected to induce near-maximal LH and progesterone secretion during the luteal phase (Lemay et al., 1982). Buserelin treatment was to be administered for a maximum of 15 days. Pregnancy was diagnosed by measuring serum hCG levels on day 13 of the luteal phase (day of first hCG/buserelin administration = day 0).

Pregnancy outcome was to be monitored. A positive pregnancy test was defined as a serum hCG level >10 mIU/ml on luteal phase day 13 or later. A clinical pregnancy was defined as an ongoing pregnancy with an amniotic sac and a positive heart beat visualized by ultrasound.

IUI was performed using a suspension of washed motile sperm cells, within 36 h of triggering ovulation.

To calculate luteal phase duration, day 1 was the first day after ovulation induction and the last day was the day before menstruation commenced.

Twenty-four patients were randomized (six in each group) and each completed the treatment according to the protocol.

Randomization process

A randomization list was computer-generated by an independent statistician. Treatment allocation instructions were placed in individually sealed envelopes to be opened at the centre in chronological order on the day of ovulation induction.

Hormone assays

Serum estradiol (E_2), serum progesterone, serum LH and serum hCG were assayed using commercially available kits at the accredited clinical centre’s central laboratory. E_2, progesterone and LH were assayed using the Elecsys 2010 system (Roche Diagnostics GmbH, Germany). The E_2 intra-assay coefficient of variation (CV) was <6% and the inter-assay CV <6%; the progesterone intra-assay CV was <3%, and the inter-assay CV <6%; the LH intra-assay CV was <2% and the inter-assay CV <5%. Serum hCG was assayed using the Beckman–Coulter system (Anablis, Belgium). The hCG intra-assay CV for a value <5 mIU/ml was <2% and the inter-assay CV <12%.

Statistical analysis

Luteal phase hormone profiles were compared between groups using the repeated measures analysis of variance method. In addition, the dose relationship was tested on late luteal phase time-points by testing the hypothesis A < B < C < D. P < 0.05 was considered statistically significant.

Results

Twenty-four eligible patients underwent mild stimulation and met the criteria for ovulation induction. On the day of randomization, the patients were similar in terms of baseline characteristics and ovarian stimulation status (Table I). All the patients developed at least one pre-ovulatory follicle, hence there was no drop-out in the study. The administration of 25 mg/day of exemestane did not significantly increase the number of pre-ovulatory follicles. All the patients were randomized and underwent IUI.

The luteal phase duration assessed in patients who did not become pregnant varied from patient to patient. It ranged between 11 and 18 days (Figure 1). All those who received buserelin ×1 every day (group D) had a luteal phase exceeding 14 days. The mean and median duration of the luteal phase was similar in the four groups (Table II).

Five positive pregnancy tests were recorded during the study. Three pregnancies were confirmed as ongoing clinical pregnancies based on the criteria defined above. No pregnancy was recorded in the hCG group (0/6), while five pregnancies were recorded in the buserelin groups (5/18; 28%).
The distribution of pregnancies in the different treatment groups is summarized in Table II.

Median serum progesterone levels for patients who did not become pregnant are presented in Figure 2. Serum progesterone levels increased in all four groups as soon as ovulation was triggered. Serum progesterone levels were similar during the first 7 days in all groups. During the second part of the luteal phase, serum progesterone levels tended to differ, with the highest levels observed in patients who received daily buserelin. On day 14 of the luteal phase, progesterone levels (median, 95% CI) were 3.0 (0.8, 5.2), 1.7 (0.5, 3.9), 3.9 (0.7, 8.5) and 7.7 (3.4, 11.9) ng/ml in groups A, B, C and D respectively (P = 0.045). Individual serum progesterone levels on day 14 of the luteal phase, which illustrate the supportive effect of the daily administration of buserelin compared to hCG or less frequent administration of buserelin, are presented in Figure 3.

Median serum E2 levels of patients who did not become pregnant are presented in Figure 4. Serum E2 concentrations were comparable during the luteal phase. The serum E2 profile was found to be statistically different in the group receiving buserelin every 2 days (group C) versus hCG controls (group A, P = 0.0126). On day 14 of the luteal phase, median LH levels were 4.2 (1.4, 7.0), 5.1 (2.2, 8.0), 4.2 (−0.3, 8.7) and 3.5 (2.4, 4.5) pg/ml in groups A, B, C and D respectively (not significant).

Buserelin treatment was well tolerated by all patients. No drop-out was recorded. Moreover, no significant adverse events were reported in terms of local or systemic tolerance. No ovarian hyperstimulation syndrome (OHSS) was noted.

Discussion
During the menstrual cycle, a normal luteal phase is required for embryo implantation and evolution of pregnancy.

Table I. Patient demographics and ovarian stimulation status on day of randomization

<table>
<thead>
<tr>
<th></th>
<th>Group A hCG (n = 6)</th>
<th>Group B Buserelin £1 every 3 days (n = 6)</th>
<th>Group C Buserelin £1 every 2 days (n = 6)</th>
<th>Group D Buserelin £1 every day (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.2 ± 1.5a</td>
<td>33.3 ± 3.8</td>
<td>30.7 ± 4.1</td>
<td>29.3 ± 4.5</td>
</tr>
<tr>
<td>Day of trigger</td>
<td>15</td>
<td>12</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>252 ± 90</td>
<td>216 ± 89</td>
<td>231 ± 66</td>
<td>242 ± 151</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.7</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Diameter of dominant follicle (mm)</td>
<td>19.0 ± 2.5</td>
<td>18.2 ± 1.6</td>
<td>19.3 ± 1.5</td>
<td>20.7 ± 1.2</td>
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$^a$Mean ± SD.
$^b$Median.
$^c$95% confidence interval.
The luteal phase is the result of intermittent stimulation of the corpus luteum by pituitary LH. During the luteal phase, pituitary LH pulses are of low frequency, leading to repeated episodes of progesterone secretion at a rate of three to five per 24 h. This contrasts with the high frequency pulsatility of LH during the follicular phase, at a rate of approximately one pulse every 90 min (Yen, 1991).

Luteal phase deficiency is a common feature of cycles resulting from stimulation of follicular development (Tavaniotou et al., 2002a). The reasons for luteal deficiency are not yet fully understood. Possible causes include induction of multiple follicle development per se, which could either directly or indirectly influence the duration of the luteal phase, supra-physiological levels of E2 that suppress endogenous LH secretion during the luteal phase, and the prolonged desensitization of pituitary gonadotroph cells by the GnRH agonist used to prevent mistimed LH rises during the follicular phase (Beckers et al., 2003).

In GnRH antagonist-treated cycles, it was initially speculated that the rapid recovery of pituitary function after antagonist cessation may render luteal supplementation redundant. However, the luteal phase is also short in GnRH antagonist-treated cycles (Albano et al., 1999; de Jong et al., 2000; Tavaniotou et al., 2002b; Beckers et al., 2003). Moreover, different pharmacological agents used as a mid-cycle LH surge substitute were recently compared and premature luteolysis was observed in all treatment groups (Beckers et al., 2003). Luteal phase duration was, however, longer with hCG than with recombinant human LH and longer than with GnRH agonist, which correlates well with the duration of the LH/hCG signal.

Luteal phase deficiency is characterized by premature regression of the corpus luteum, resulting in a shortened phase (<10 days), low serum progesterone levels and a delayed secretory transformation of the endometrium (Smitz et al., 1993). The consequences of luteal phase deficiency are a decreased embryo implantation rate, a lower pregnancy rate and an increased miscarriage rate when pregnancy is established (Pritts and Atwood, 2002).

Table II. Characteristics of the luteal phase and pregnancy in patients treated with hCG or buserelin

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
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</thead>
<tbody>
<tr>
<td>HCG (n = 5)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Luteal phase duration</td>
<td>14.7 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 1.7</td>
<td>14.8 ± 3.3</td>
<td>15.3 ± 0.6</td>
</tr>
<tr>
<td>in non-pregnant patients (days)</td>
<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>No. of patients</td>
<td>4/6</td>
<td>2/5</td>
<td>2/4</td>
<td>4/4</td>
</tr>
<tr>
<td>with a luteal phase = 15 days</td>
<td>14, 15.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.5, 15.5</td>
<td>11.8, 18.2</td>
<td>14.4, 15.6</td>
</tr>
<tr>
<td>Corpus luteum diameter</td>
<td>20.0 ± 14.8</td>
<td>18.0 ± 2.9</td>
<td>18.5 ± 5.7</td>
<td>22.4 ± 8.5</td>
</tr>
<tr>
<td>on luteal phase day 7 (mm)</td>
<td>0/6</td>
<td>1/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
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<tr>
<td>Clinical pregnancy</td>
<td></td>
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</table>

<sup>a</sup>Mean ± SD.
<sup>b</sup>Median.
<sup>c</sup>95% confidence interval.

Figure 2. Luteal phase progesterone levels in non-pregnant patients: median (A, hCG (n = 6); B, buserelin 100 μg every 3 days (n = 5); C, buserelin 100 μg every 2 days (n = 4); D, buserelin 100 μg every day (n = 4)).

Figure 3. Individual serum progesterone levels in non-pregnant patients (closed circle) and pregnant patients (open circles) on day 14 of the luteal phase and median values.
Luteal phase estradiol (E₂) levels in non-pregnant patients (for ‘n’ see figure 2).

Figure 4. Luteal phase estradiol (E₂) levels in non-pregnant patients (for ‘n’ see figure 2).

Luteal phase LH levels: median

Figure 5. Luteal phase LH levels in non-pregnant patients (for ‘n’ see figure 2).

Luteal phase supplementation or support is therefore common practice in infertility treatment to significantly improve embryo implantation rates, clinical pregnancy rates and delivery rates (Pritts and Atwood, 2002).

Native GnRH has been used to support the luteal phase when inducing ovulation in hypogonadotrophic hypogonadal patients (World Health Organization group I anovulation) (Homburg et al., 1989). However, it requires i.v. or s.c. administration of GnRH every 60–120 min using a portable pump. This is not seen as convenient for routine use. Using a GnRH agonist with a prolonged duration of activity would therefore appear to be a viable option. The clinical challenge is, that GnRH super-agonists tend to rapidly induce desensitization of pituitary gonadotroph cells (Loumaye, 1990). Indeed, 20 years ago, GnRH agonists were tested as possible agents to induce luteolysis for contraceptive purposes. One or two administrations of 500 μg of buserelin during the luteal phase were shown to slightly impair the luteal phase (Lemay et al., 1982, 1983). This effect was not further investigated, however.

Because desensitization is related to the extent and the dose of exposure, we postulated that, as long as an adequate dose and frequency of administration is used, GnRH agonist may actually maintain its stimulatory effect through the luteal phase, and hence restore serum LH levels and support the luteal phase. We thus selected a dose which has been shown to induce near-maximal LH and progesterone secretion during the luteal phase after a single administration, i.e. 100 μg intranasally (Lemay et al., 1982), and to adhere to a low frequency of administration, i.e. not more than once a day. Nevertheless, the risk of failure to support the luteal phase was deemed significant and it was therefore decided to first test this concept in patients undergoing treatment less invasive than assisted reproductive treatment, i.e. an IUI cycle.

Our study shows that exemestane did support the development of a pre-ovulatory follicle in all patients, but did not increase the number of pre-ovulatory follicles. This contrasts with previous reports using different aromatase inhibitors at different doses (Mitwally and Casper, 2002, 2004). In our study, ovulation was triggered in all patients. Different doses and different durations of administration would need to be tested, however, before reaching any conclusion on the ability of this specific aromatase inhibitor to stimulate multiple follicular development (Casper, 2003; Healy et al., 2003).

Regarding the luteal phase, the first striking observation to be made was that, at the dose and all the frequencies of administration used, buserelin did not appear to shorten the luteal phase, compared to the control group. Moreover, at a dose of 100 μg a day, the luteal phase consistently lasted >14 days and the late luteal progesterone serum levels were significantly increased. This contrasts with a previous report, in which nafarelin was used from the mid-luteal phase onwards after stimulation with clomiphene citrate and ovulation induction with hCG (Schmidt-Sarosi et al., 1995). In that study, significant signs of luteal deficiency were recorded. The peptide used, the dose, the timing and the frequency of administration were very different from our study.

The second important observation is that, although the number of patients was small, all the pregnancies were obtained in the buserelin groups and two out of three clinical pregnancies were recorded in the daily dose group. This indicates that daily administration of buserelin during the luteal phase does not prevent pregnancy, corroborating reports of spontaneous pregnancies occurring in cycles during which GnRH agonist was inadvertently administered in a conception cycle prior to an assisted reproduction cycle (Ron-El et al., 1990; Smitz et al., 1991; Golan et al., 1992; Wilshire et al., 1993; Young et al., 1993; Elefant et al., 1995; Gartner et al., 1997; Chardonnens et al., 1998). Notably, the administration of one dose of a GnRH agonist during the luteal phase of an assisted reproduction cycle was recently shown to increase the pregnancy rate in recipients of donated oocytes (Tesarik et al., 2004). Because this was observed after a single administration of triptorelin, and trophoblast cells express GnRH receptors, the authors hypothesized a direct beneficial effect on the embryo (Raga et al., 1998). A direct beneficial effect on the endometrium could, however, also be considered since GnRH receptors are expressed in human endometrium (Raga et al., 1998).

E₂ levels are also of interest. In this study, a significant difference was observed between groups. In patients receiving buserelin only once every 3 days, luteal E₂ levels were
lower than in hCG-treated patients. This is in accordance with the observation that very low levels of E2 and luteolysis are observed in a non-supplemented luteal phase following ovulation induction with a GnRH agonist (Kol, 2004). By contrast, in our study, the serum E2 levels of patients receiving buserelin every day did not differ from the controls. Our study therefore shows that after ovulation has been triggered with a GnRH agonist, no luteolysis occurs if the analogue administration is continued throughout the luteal phase at sufficient frequency.

Serum LH levels were high after ovulation induction, and then declined throughout the luteal phase. LH levels, however, remained detectable in all patients at all time-points (serum LH range: 1.6–13.3 IU/l). There was no significant difference between treatment groups. Blood tests performed in the morning following the previous evening’s intranasal administration of buserelin did not allow measurement of the acute release of LH, which peaks around 4 h after nasal administration (Lemay et al., 1982).

The safety of administering a GnRH analogue during the luteal phase of a conception cycle has to be addressed. According to the manufacturer, buserelin and some other GnRH agonists have been tested in standard toxicology studies, which include a teratogenicity assessment. The peptides were not found to be teratogenic (Suprefact Patient Information Leaflet). In addition, inadvertent administration of a GnRH agonist during a conception cycle has been reported by numerous groups (Ron-El et al., 1990; Smits et al., 1991; Golan et al., 1992; Wilshire et al. 1993; Young et al., 1993; Elefant et al., 1995; Gartner et al., 1997; Chardonnens et al., 1998). The pregnancy outcome did not raise concerns. Finally, long-acting depot preparations of GnRH are widely used in assisted reproduction to prevent premature LH surges. Considering its very long half-life, it is obvious that significant peptide exposure is encountered during the luteal phase. It can therefore be concluded that continuing GnRH agonist administration during the embryo peri-implantation period does not appear to carry a risk for the embryo. Current observations are thus reassuring and in line with previous observations. However, a much larger sample size is needed to establish the absolute safety of the proposed luteal support.

In conclusion, this study suggests that testing the use of a GnRH agonist as luteal support in assisted reproduction appears feasible. Indeed, it would offer significant advantages over current treatments in patients undergoing ovulation induction and in assisted reproduction patients when a GnRH antagonist is used to prevent a mistimed LH surge. Ovulation induction in polycystic ovaries is frequently associated with a premature LH surge and OHSS. Triggering an LH surge with a GnRH agonist has been well documented and it is used in some centres to reduce the risk of OHSS. Our proposed new regimen for luteal support would potentially improve their practice. It may be safer and more convenient to use than current therapies. It would reduce significant serum LH levels which would be of proven benefit since, beyond maintaining progesterone and E2 levels, this would stimulate other peptides secreted by the corpus luteum, such as relaxin (Lounaye et al., 1994). In addition, a direct beneficial effect of LH on the endometrium is currently being debated (Stewart, 2001; Rao and Lei, 2002; Tesarik et al., 2003), which could include stimulation of angiogenic and growth factors, as well as cytokines involved in implantation (Licht et al., 2001). Finally, GnRH itself could have a direct beneficial effect on embryo development potential (Tesarik et al., 2004).

The extrapolation of these data to assisted reproduction patients in whom a premature LH surge is prevented by administration of a GnRH antagonist remains to be demonstrated. However, this positive proof-of-concept study supports the testing of this novel luteal phase treatment in such patients.

References


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