JUNE 1990 VOL. 33 NO. 6

THERIOGENOLOGY

AN INTERNATIONAL JOURNAL OF

ANIMAL REPRODUCTION

VICTOR M. SHILLE
Editor

Butterworth–Heinemann

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Volume 33, Number 6, June 1990

(continued)

THE EFFECT OF A POTENT GnRH AGONIST ON GONADAL AND SEXUAL ACTIVITY IN THE HORSE

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Received for publication: *October 25, 1989*
Accepted: *March 6, 1990*

ABSTRACT

A potent agonist of gonadotropin releasing hormone (GnRHa) was administered to stallions (n=3) and mares (n=4) during the physiologic breeding season in order to downregulate gonadal activity. Stallions received an increasing regimen of 12.5 mg (5 d), 25 mg (10 d), and 50 mg (15 d) GnRHa per day, for a total of 30 d. Libido and semen quality were not affected by the treatment. The only treatment effect observed was an initial rise in luteinizing hormone (LH) concentration and a slight decline in testosterone concentration during treatment in two of three stallions. These data suggest that daily GnRHa therapy is not a practical method for the suppression of sexual behavior in the stallion at the dosage and interval utilized.

Mares received 25 mg i.m. GnRHa per day, for a total of 35 d (n=2), or 40 d (n=2). While estrus and ovulation occurred in two mares during the first half of treatment, follicular development was suppressed in all mares by the end of treatment. Following treatment, ovulation occurred in all mares between 12 and 16 d after treatment. While gonadal activity and estrous behavior can be suppressed in mares with GnRHa therapy, animals require up to 30 d of treatment before suppression of ovarian activity is achieved.

Key words: GnRH-agonist, downregulation, sexual behavior, mare, stallion

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Acknowledgments

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We wish to thank the Salk Institute for providing the GnRH-analogue and Ms. Coralie Munro, Ms. Nicole Janssen and Dr. J. Roser for the analysis. This project was supported by the Equine Research Laboratory with funds provided by the Oak Tree Racing Association and the State of California Satellite Wagering Fund.

INTRODUCTION

Downregulation refers to the paradoxical decrease of activity of an organ in response to constant stimulation. Continuous exposure of the pituitary gland to constant high concentrations of GnRH, or a potent GnRH agonist, results in a decrease of pituitary responsiveness to GnRH due to a decrease in available GnRH receptors (1). This effect leads to a decrease in plasma concentrations of LH and follicle stimulating hormone (FSH) and, consequently, a decrease in gonadal steroid production. GnRH, and associated agonists, may also have a direct inhibitory action at the level of both the pituitary gland and the gonads (2-4). Receptors for GnRH have been demonstrated in the testis and ovary (5).

GnRH agonists have been used in men for the treatment of precocious puberty and cancer of the prostate gland, with treatment of adult men having a suppressive effect on libido (6-8). In women, GnRH agonists have been used to suppress ovarian cyclicity as well as to treat steroid-mediated diseases (9-10). Gonadotropin releasing hormone, GnRH agonists, or antibodies to GnRH, have been used to downregulate or block GnRH receptors and, thus, block the preovulatory gonadotropin surge (and ovulation) as a means of contraception in mammals (11-13). The end result is an inhibition of follicle growth, reduced estrogen secretion, and involution of the ovaries and uterus.

The aim of our study was to investigate the ability of a potent GnRH agonist to inhibit reproductive activity in stallions and mares. The GnRH agonist was administrated to normal stallions and mares during the breeding season to evaluate the use of GnRH agonist therapy as a means to downregulate gonadal activity and thus control sexual behavior in the competing horse.

MATERIALS AND METHODS

GnRH agonist

A potent GnRH agonist[a] (GnRHa) was diluted in physiologic saline so that the total volume was 1 ml per injection regardless of the dosage. Stallions were given 12.5 mg GnRHa per day for 5 d, then 25 mg per day for 10 d and finally 50 mg per day for 15 d. Mares were treated with 25 mg GnRHa per day, with treatment initiated during the luteal phase of the estrous cycle, 8 d after ovulation. Mares 1 and 4 were treated for 40 d, while Mares 2 and 3 were treated for 35 d. The daily dosage was divided and administered intramuscularly at 12-h intervals.

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[a] Des-Gly^{10}-D-Try^{6}-Pro-Ethylamide-LHRH, Bachem, Los Angeles, CA.

Stallions

Three adults stallions, 3, 9 and 10 yr of age, with normal fertility and libido, were used between March and October. Prior to treatment, semen was collected at least four times with an artificial vagina[b] and evaluated using standard procedures (Guidelines of the Society of Theriogenology). The evaluation of libido was based on time from first visual contact of the stallion with the mare to erection, and time from mounting to ejaculation. During GnRHa treatment, libido was evaluated and semen was collected once a week; the examination was repeated within one week following the end of treatment.

Blood was collected in heparinized tubes[c] by jugular venipuncture daily from 10 d prior until 5 d after GnRHa treatment. Plasma was stored frozen (-20 °C) until analyzed for LH and testosterone (T) concentrations.

Mares

The four mares used in this study had previous normal interovulatory intervals (20 to 22 d). They were then monitored through one estrous cycle prior to treatment with GnRHa. The mares were observed for sexual receptivity by teasing with a stallion, and the genital tract was examined by palpation and ultrasonography[d] per rectum on a daily basis during estrus and every other day during diestrus.

Heparinized blood was obtained daily by jugular venipuncture starting with the ovulation before treatment and continuing until the first ovulation after treatment. Plasma was stored frozen (-20 °C) until analyzed for LH, estrogen conjugate (EC) and progesterone (Po) concentrations.

Hormone analysis

Plasma progesterone concentrations were determined by enzyme-immunoassay (14). The progesterone antibody cross-reacted with 11α-hydroxyprogesterone (21.4%), 5α-pregnane-3,20-dione (29.5%), and 20β-dihydroprogesterone (2.4%) while the cross-reaction was <0.5% for 17α-hydroxyprogesterone, estradiol-17α, cortisol, Δ^4-androstenedione and testosterone. The sensitivity of the assay was 2.5 pg/ml. The inter-assay coefficient of variation ranged from 1.2 to 4.3% at different concentrations of the hormone, and the intra-assay coefficient of variation ranged from 2.7 to 8.4%.

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[b] Missouri-type artificial vagina, Nasco, Modesto, CA.

[c] Monoject, Sherwood Medical, St Louis, MO.

[d] Aloka 210, 5 MHz linear transducer, Corometrics Medical Systems Inc, North Wallingford, CT.

Estrogen conjugate concentrations in plasma were determined with a direct radioimmunoassay (RIA). The antibody (R-583) was produced in rabbits and was directed against estrone-3-glucuronide which had been conjugated to bovine serum albumin. The antiserum was used at a dilution of 1:8000 in Tris buffer (0.1 M Tris pH 8.4, 0.1% gelatin, 0.9% NaCl, 0.1% sodium azide). When using estrone-3-sulfate as the standard (100%), the antibody cross reacted with estrone (200%), estradiol 17-β (100%), equilin (50%), estrone-3-glucuronide (38%), estradiol-3-sulfate (21%), and estradiol-3-glucuronide (6.8%); the cross reaction was less than 0.5% with all nonestrogenic steroids tested. The limit of the sensitivity was 0.018 ng/tube or 0.36 ng/ml plasma. The inter-assay coefficients of variation were 13% (n=10) to 21% (n=15) at 20 to 30% binding and 10% (n=15) to 12% (n=9) at 50 to 60% binding on the standard curve. The general assay protocol has been described previously (15-16).

Plasma T concentrations were measured by radio-immunoassay (17). The antibody was raised in sheep to testosterone-11-BSA.[e] The cross-reactivity when using testosterone (100%) as the standard was as follows: dihydrotestosterone 33%; 5α-androstan-3α,17β-diol 10%; 5α-androstan-3β,17β-diol 5%. The inter-and intra-assay coefficients of variation were 6.7 and 5%, respectively, and 1 pg could be differentiated from zero.

Plasma LH concentrations were determined by radio-immunoassay (18). The LH antibody used was a monoclonal antibody (518B7) generated against bovine LH. The antibody cross-reacted with purified equine FSH (1.4%) and TSH (3.8%). The sensitivity of the assay was 1 pg/ml. The intra- and inter-assay coefficients of variation were 4.4 and 6.6%, respectively.

RESULTS

Stallions

No change in either sexual behavior or semen characteristics was observed in response to GnRHa treatment. Spermatozoal motility, morphology and volume did not vary more than ± 15% from the pretreatment values. One stallion (Stallion C) was bred to a mare on the first day after the end of treatment, with the breeding resulting in pregnancy.

Stallion A. In Stallion A (Figure 1), the mean pretreatment T concentration was 260 ± 145 pg/ml (mean ± SD; range 52 to 419 pg/ml). The T concentrations increased to a peak of 1,900 pg/ml on Day 2 of treatment and then declined

[e] Courtesy of Dr. G. Niswender, Colorado State University, Fort Collins, CO.

sharply to Day 6. The T values then declined slowly during treatment from 675 pg/ml on Day 7 to a low of 61 pg/ml at the end of treatment. While LH concentrations decreased slowly during treatment, values were within two standard deviations of the pretreatment mean (2.0 ± 0.9 ng/ml; range, 0.7 to 3.2 ng/ml) during the entire period. The LH treatment concentrations ranged from 3.1 ng/ml on Day 7 to 0.4 ng/ml on the next to last day of treatment.

Stallion B. In Stallion B, the mean pretreatment T concentration was 845 ± 213 pg/ml (range, 594 to 1236 pg/ml). The T values increased to a peak of 2,412 pg/ml on Day 2 of treatment, which was followed by a sharp decline as in Stallion A. From Day 5 to the end of treatment, T values remained within the pretreatment range of 560 to 1702 pg/ml. While LH treatment values (2.3 ± 0.4 ng/ml; range, 1.4 to 3.8 ng/ml) tended to be slightly higher than pretreatment LH concentrations (1.7 ± 0.5 ng/ml; range, 1 to 2.6 ng/ml), the differences were not significant.

Stallion C. In Stallion C, the mean pretreatment T concentration was 808 ± 223 pg/ml (range, 668 to 1243 pg/ml). The T values increased to peak at 3,961 pg/ml on Day 2 of treatment, which was followed by a decline through Day 5. The T values for the rest of the treatment period (range, 120 to 1740 pg/ml) were not different from the pretreatment mean. The mean pretreatment LH concentration was 5.0 ± 0.6 ng/ml (range 4 to 5.9 ng/ml). While an initial elevation in LH concentration was observed on Day 2 of treatment (7.0 ng/ml), values during the rest of treatment were significantly lower (0.6 to 2.3 ng/ml; $P<0.05$) than pretreatment values.

Mares

In all mares, GnRHa treatment resulted in suppression of ovarian activity, with only small follicles (<20 mm) present at the end of the treatment periods of 35 or 40 d. While ovulation occurred during the first half of treatment in two mares, suppression of ovarian activity resulted in lengthened interovulatory intervals in all mares, which ranged from 35 to 54 d. Ovulation occurred within 12 to 16 d after the end of treatment in all four mares.

Mare 1. In Mare 1 (Figure 2a), the GnRH agonist treatment caused a four-fold increase in LH concentrations within 24 h. This was followed by a return to the pretreatment value by Day 6, and LH values subsequently declined to about 1 ng/ml by Day 9, remaining at this level for the duration of treatment. The LH concentrations, barely detectable for the first three days post treatment, began to increase above the 1 ng/ml level at about Day 8 post treatment, with a preovulatory surge occurring through Day 16. Ovulation occurred on Day 16, in conjunction with the posttreatment LH surge, with GnRHa treatment causing an interovulatory interval of 54 d.

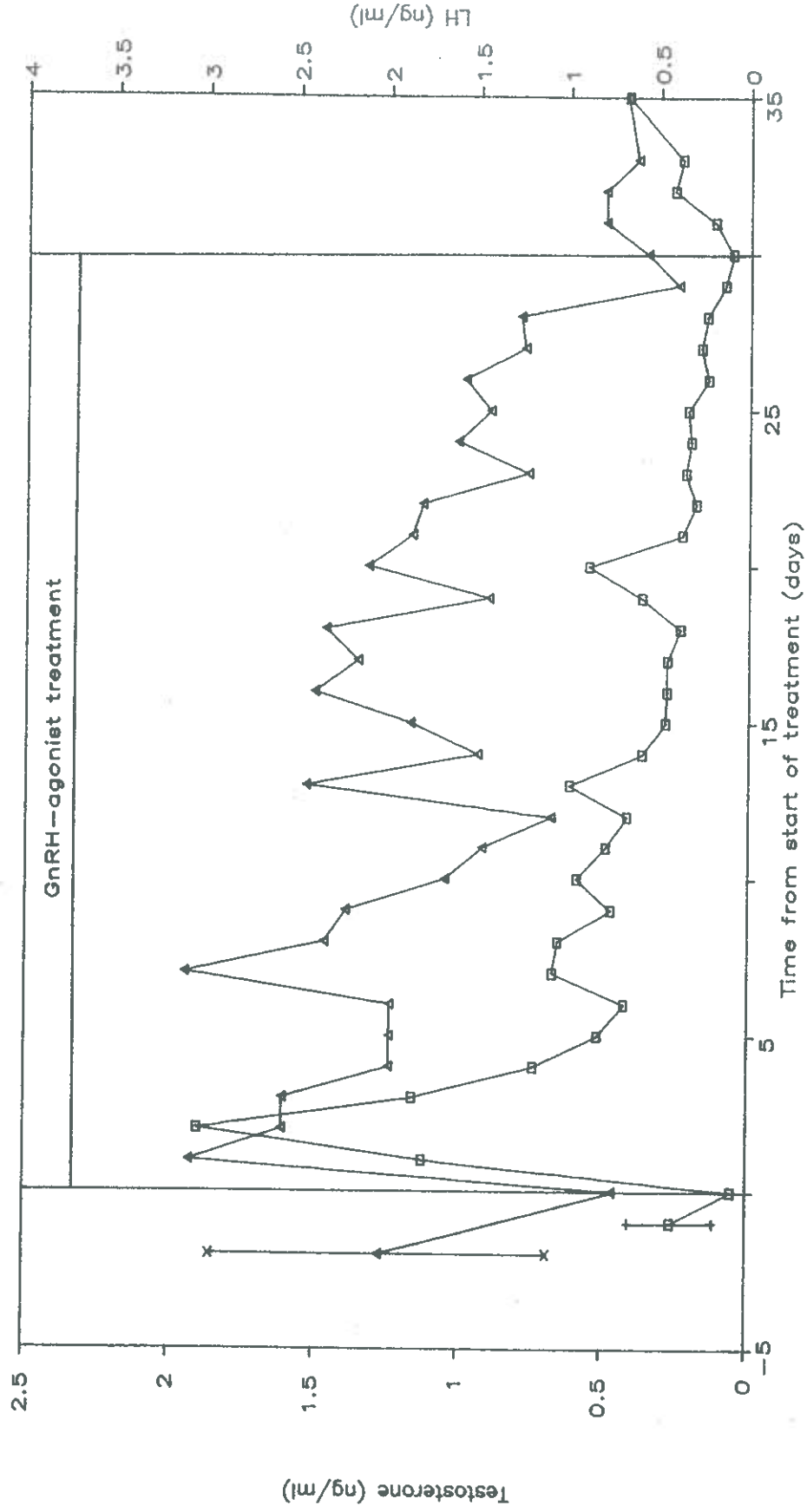


Figure 1. Effect of GnRH-agonist on LH (Δ) and testosterone (□) concentrations in Stallion A. Pretreatment values are represented as mean ± one standard deviation.

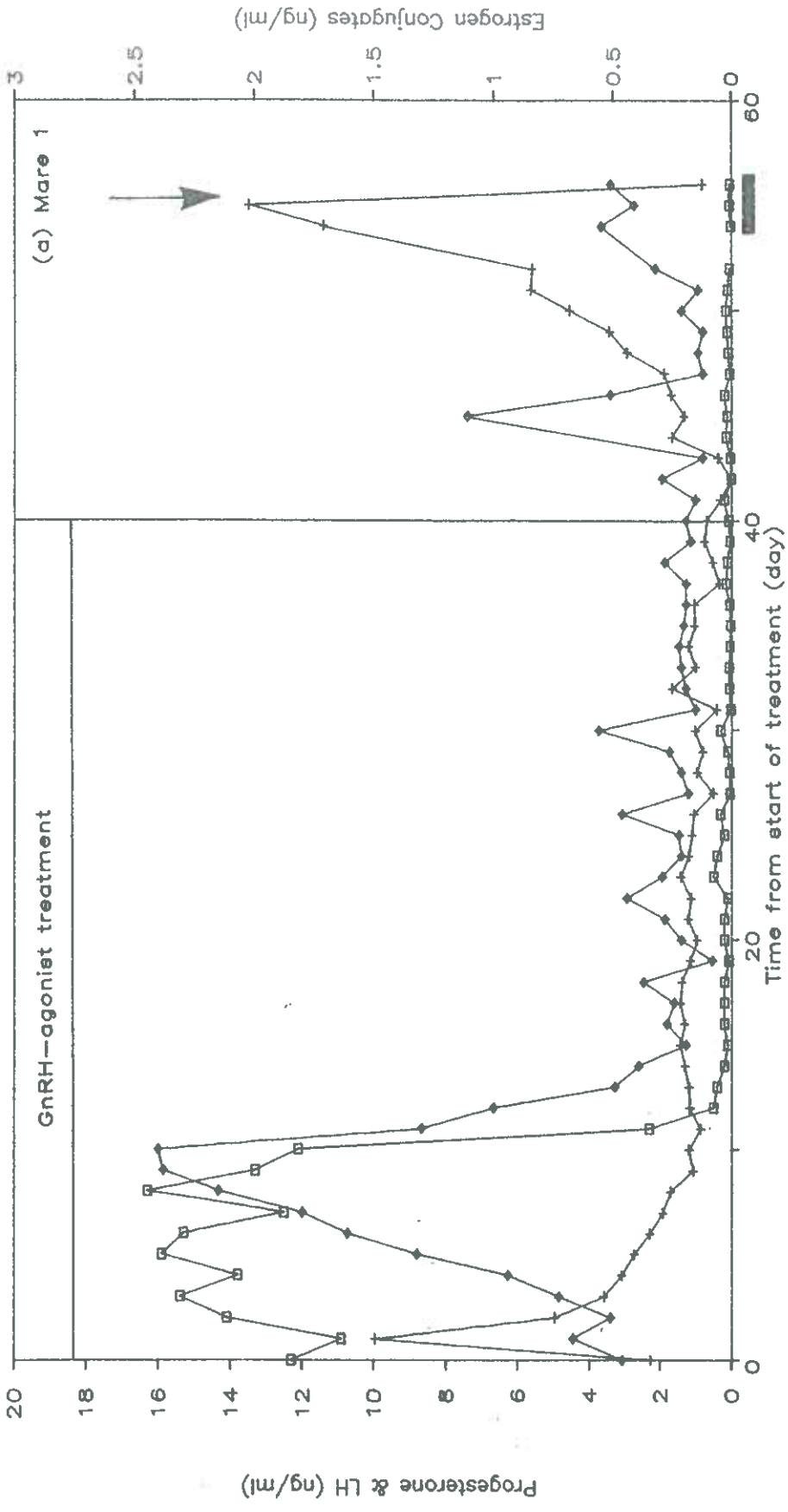
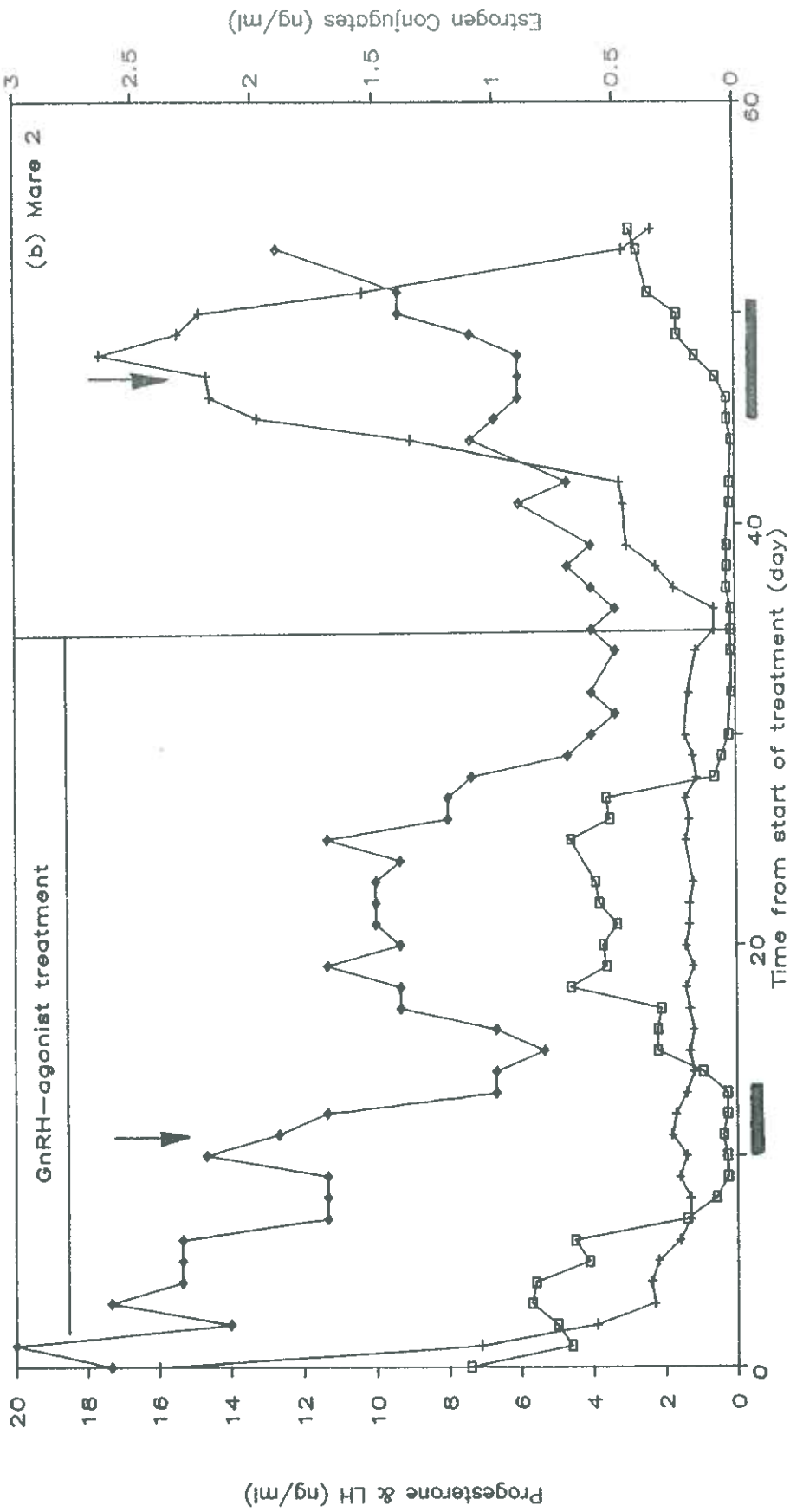


Figure 2. Effect of GnRH-agonist on progesterone (□), estrogen conjugate (◇) and LH (+) in Mares 1 through 4. Vertical arrows indicate time of ovulation and horizontal bars along the X-axis indicate estrous behavior.



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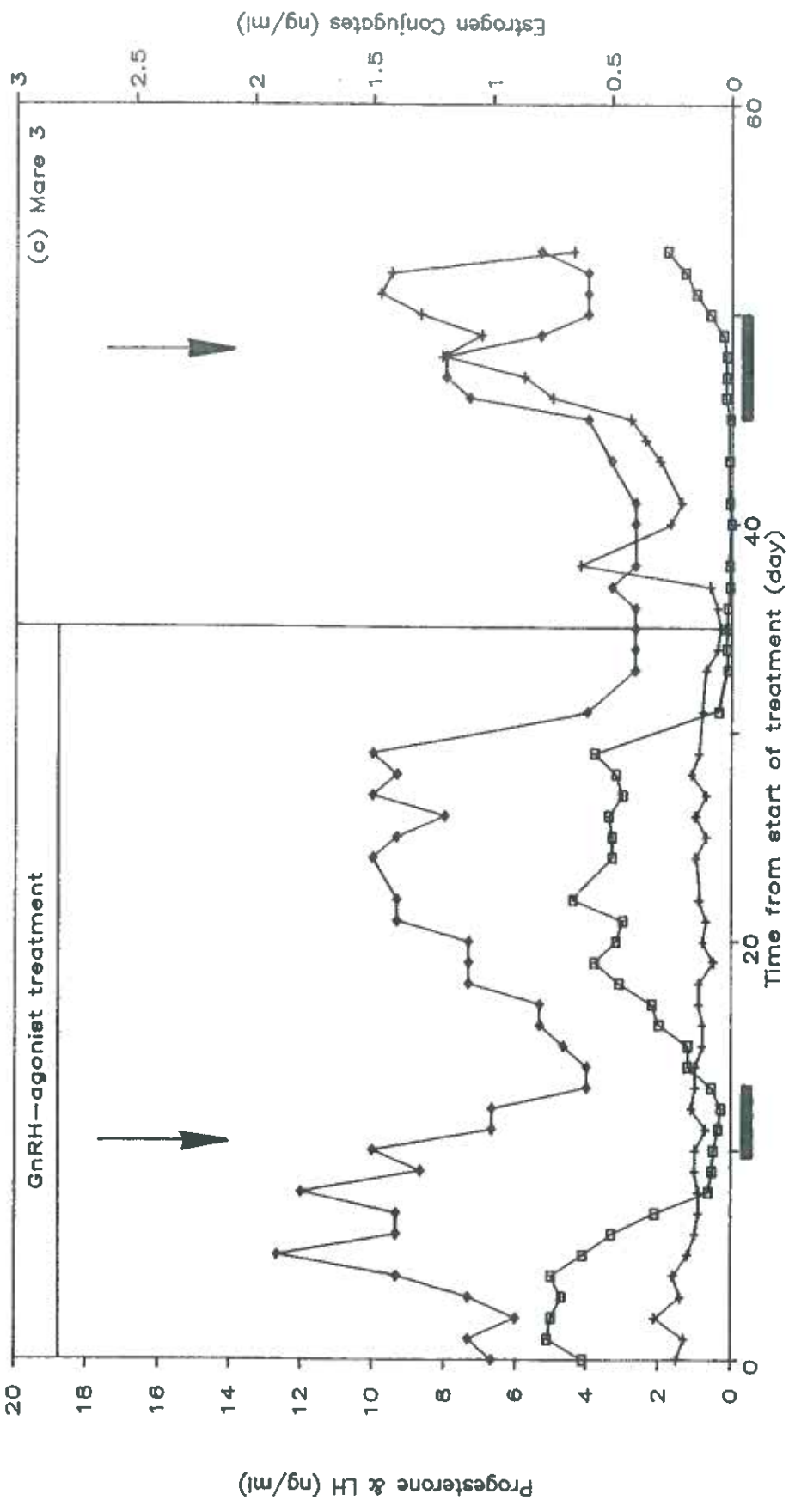

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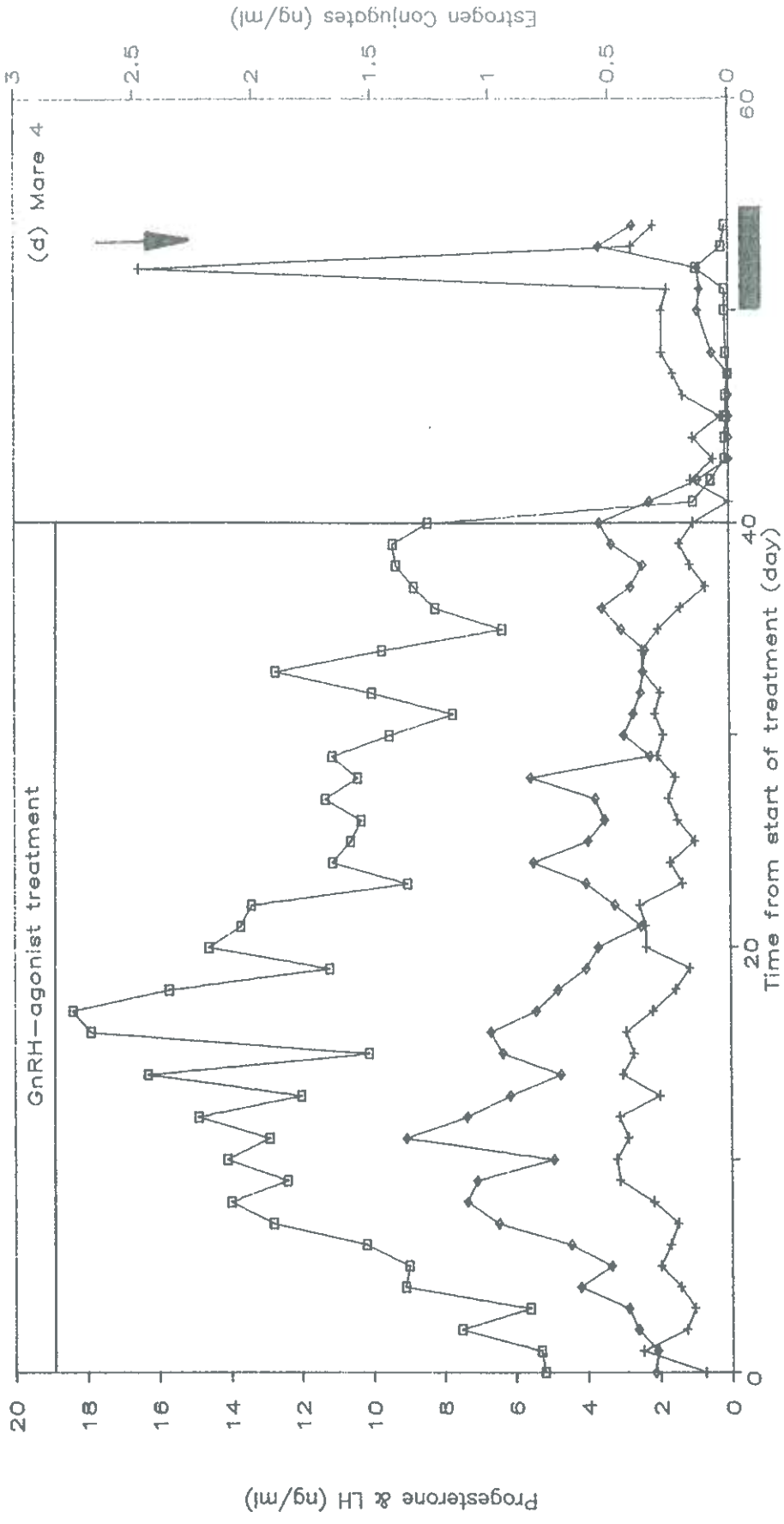

Figure 2. (continued)

Estrogen conjugate concentrations began to increase 4 d after the onset of treatment, reaching a peak value at Day 10 of treatment. This was followed by an abrupt decline in EC values over the next 5 d. Values then remained low during treatment. Five days after cessation of treatment, one high value was observed, followed by a preovulatory increase between Days 12 and 16. The duration of the luteal phase in which treatment was initiated was 18 d. Following luteolysis, progesterone concentrations were then low (<0.4 ng/ml) during the rest of the treatment regimen and through the first posttreatment ovulation.

Sexual receptivity in Mare 1 was not observed during treatment and did not occur until Days 14 through 17 post treatment, resulting in a 60-d period with no estrous behavior.

Mare 2: In Mare 2 (Figure 2b), LH concentrations decreased from 16 ng/ml at the start of treatment to about 2 ng/ ml by the third day of treatment. From Day 6 through the duration of the treatment regimen, LH values were between 1 and 2 ng/ml. Subsequently, LH values began to increase on the second day post treatment, with a peak value of 17.6 ng/ml observed on Day 13.

Ovulation occurred on Day 12 of treatment (20-d ovulatory interval) and then not again until 12 d after treatment (35-d ovulatory interval). Ovarian activity was suppressed, as indicated by EC values, following regression of the corpus luteum through the end of treatment. The duration of the luteal phase initiated before the onset of treatment was 14 d and the posttreatment luteal phase was 15 d. Luteal activity was normal in both cases as indicated by progesterone concentrations.

Estrogen conjugate concentrations were high at the beginning of the treatment regimen and then declined until Day 15. Values then rose in the preovulatory period at treatment Days 9 to 12 and remained elevated until Day 29 of treatment. Estrogen conjugate values decreased around the time of luteolysis and then remained low until about Day 6 post treatment, when they began to progressively increase through Day 18 post treatment.

Sexual receptivity in Mare 2 was observed on Days 10 to 13 of treatment and again 42 d later, on Days 8 to 13 after treatment.

Mare 3: In Mare 3 (Figure 2c) the response was similar to that in Mare 2. Ovulation occurred on Day 13 of treatment at an ovulatory interval of 21 d. The subsequent ovulatory interval was 37 d, with ovulation occurring on Day 14 after treatment. The initial luteal phase was 15 d, and the subsequent luteal phase (initiated during treatment) was 16 d in duration. Estrus was observed in conjunction with both ovulatory periods.

Mare 4: In Mare 4 (Figure 2d), LH concentrations ranged from 0.8 and 3.2 ng/ml during the period of treatment. The LH concentration was below detectable levels on the day after the end of treatment and was less than 2 ng/ml until Day 13 after treatment, when a single elevated value of 16.7 ng/ml was observed 1 d prior to ovulation.

Progesterone values indicated the mare had a persistence of the luteal phase during the period of treatment. There appeared to be enhancement of progesterone production during treatment, with highest values noted on Days 16 and 17; values then tended to decrease throughout the pretreatment period. The luteal phase ended abruptly the day after the end of treatment.

Estrogen conjugate concentrations increased over the first 10 d of treatment, then declined slowly during the rest of the treatment regimen. Estrogen conjugate concentrations declined to undetectable concentrations by Day 3 after treatment. The increase in EC concentrations on Days 13 and 14 after treatment was associated with the development of a Graafian follicle and ovulation on Day 14.

Sexual receptivity in Mare 4 was observed on Days 10 to 15 after treatment in conjunction with ovulation.

DISCUSSION

Stallions

The secretory pattern of the major hormone responsible for male-sexual behavior, testosterone, was not inhibited by GnRH agonist administration and no changes in libido were observed (19). The dynamics of testosterone secretion were similar for all three stallions. Testosterone concentrations peaked early during treatment, indicating an effect of the GnRH-agonist on gonadal steroid secretion, but then decreased to pretreatment values within 4 d of treatment. In two of three stallions, the increase in testosterone secretion at the beginning of treatment did not coincide with a perceptible rise in LH concentrations, in that LH concentrations were fairly constant throughout the experimental period in all three animals. This is surprising as LH is considered to be the pituitary gonadotropin that stimulates gonadal testosterone production (20). This apparent incongruity could be due to a direct action of the GnRH-agonist on the testis, as has been indicated in previous studies (2-4). On the other hand, this could indicate that the assay system used in our study did not reflect changes in the bioactivity of LH which were responsible for the early testosterone rises.

In one stallion (Stallion A), testosterone secretion tended to decrease during treatment and increase after treatment. Thus, it is possible that prolonged treatment would inhibit testosterone production and attenuate libido, but the mode of administration and the cost of the GnRH-agonist prohibit its use over a long

period of time. Because testosterone concentrations did not decrease in our study, the maintenance of spermatogenesis, is not surprising. Fertility was not adversely altered, at least in one stallion, as illustrated by the successful breeding of a mare on the first day after the end of treatment.

Mares

Two types of responses were observed in mares treated with GnRH agonist. In the first type (Mare 1, Figure 2a), ovarian activity was sufficiently suppressed to inhibit ovulation during treatment. In the second type of response (Mare 2, Figure 2b and Mare 3, Figure 2c), folliculogenesis and ovulation were not initially inhibited. Both mares ovulated between 10 and 15 d after the onset of treatment at normal interovulatory intervals of 20 and 21 d. Subsequently, follicle activity was suppressed and ovulation did not occur until between 10 and 15 d after the end of treatment.

The reason ovulation was not inhibited in Mares 2 and 3 by GnRH agonist treatment after the first luteal, is not obvious. It is possible that GnRHa is only able to block folliculogenesis if treatment is initiated before the next ovulatory follicle has been selected. Once the ovulatory follicle reaches a certain stage in its development, GnRHa treatment may not be able to impede development and the follicle may be able to progress to the point of ovulation with minimal gonadotropin support. Thus, treatment in Mares 2 and 3 may have begun after the ovulatory follicle was selected, whereas in Mare 1 it was initiated before selection of the ovulatory follicle. Sirois et al. (21) reported that the ovulatory follicle in the mare can be recruited as early as Day 5 or 6 post ovulation, a time that preceded treatment in Mares 2 and 3.

In all mares, ovulation did not occur until 12 to 16 d after the end of treatment, indicating that ovarian activity was inhibited during treatment. The time interval from the end of treatment to ovulation is analogous to the time required to stimulate ovulation, 9 to 20 d, in mares in deep anestrus (22-24).

Retrospectively, it was determined that the pretreatment ovulation in the fourth mare (Mare 4, Figure 2c) was a luteal phase ovulation. A persistent luteal phase developed either before or during the period of treatment. We do not think that the treatment was responsible for the initial development of persistence of the corpus luteum in that the three other mares in the study underwent luteal regression at the expected time. The syndrome of the prolonged luteal phase is relatively common in nonpregnant mares and can originate from an inability of the uterus to synthesize and release PGF_{2a} in quantities sufficient to cause luteolysis, and not because of enhanced luteotropic activity (25,26). The luteal phase in Mare 4 appeared to be supported by the GnRH agonist treatment, in that LH and progesterone concentrations remained elevated, then declined precipitously after treatment (8.4 ng/ml at 12 h after the last GnRHa injection and 1 ng/ml 24 h later)

in conjunction with complete luteolysis. The progesterone decline occurred in conjunction with a decline in LH concentrations from 1 ng/ml at 12 h after the last GnRHa injection to non-detectable concentrations 24 h later.

While we do not have definitive evidence for the origin of estrogen secretion during the luteal phase, it is striking that the decline in EC concentrations generally occurs in parallel with the regression of the corpus luteum, as defined by progesterone concentrations. The apparent parallelism between estrogen and progesterone secretion that can be seen in the four mares suggests that both may be secreted by the same source, the corpus luteum. Estrogen conjugate concentrations do consistently increase prior to ovulation in the absence of luteal tissue, which is a clear indication of a follicular source at this time.

Estrogen conjugate concentrations in Mare 2 (Figure 2b) and Mare 3 (Figure 2c), failed to decline at the end of the first luteal phase during treatment with GnRH agonist. The pattern we have obtained in other, untreated, mares (unpublished data) is for EC values to decline in parallel with progesterone values at the time of luteolysis. The continuation of high EC values immediately following regression of the corpus luteum suggests that follicle activity may have been promoted by the GnRH agonist treatment during the luteal phase; EC values did decline at the time of ovulation. At the end of the second luteal phase in Mares 2 and 3, when depression of follicle activity had occurred, EC values declined in concert with luteolysis.

The development of follicles through ovulation occurred in the presence of relatively low LH concentrations. We have previously found that ovulation can occur in the mare during the luteal phase of the cycle at a time when LH concentrations are relatively low (27). This brings into question the exact role of LH in ovulation in the mare; LH is likely important for ovulation, but with a less precise relationship than is usually observed in other domestic animals.

It appears that GnRHa is not a practical treatment for the control of sexual behavior in the horse. Treatment of stallions for 30 d did not affect either their libido or semen quality. Ovarian activity, and thus sexual activity, can be suppressed in the mare, but it requires up to 30 d of treatment to effect. In the mare, alternative treatments such as progestogens have been shown to suppress estrous behavior and ovarian activity in a more predictable manner (28-29). In the stallion, progestogen treatment may also be effective in the control of sexual behavior, but this needs further investigation.

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