

# Induction of luteal regression in the marmoset monkey (*Callithrix jacchus*) by a gonadotrophin-releasing hormone antagonist and the effects on subsequent follicular development

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**Summary.** Doses of 100 or 200 µg of a novel GnRH antagonist ([N-acetyl-DβNal1-D-pCl-Phe2-D-Phe3-D-Arg6-Phe7-Arg8-D-Ala10]NH<sub>2</sub> GnRH) (4 animals/dose) were administered on Days 10/11 of the luteal phase and induced a marked suppression of circulating bioactive LH and progesterone concentrations within 1 day of treatment ( $P < 0.01$ ). Thereafter, progesterone concentrations remained low or undetectable until after the next ovulation. Similar results were obtained when 200 µg antagonist were given on Days 5/6 of the luteal phase ( $N = 4$ ). The interval from injection of antagonist (200 µg but not 100 µg) to ovulation (based on a rise in progesterone above 10 ng/ml) was significantly longer than that from prostaglandin-induced luteal regression to ovulation in control cycles ( $N = 4$ /treatment) (range, 13–15 days after antagonist vs 8–10 days after prostaglandin,  $P < 0.01$ ). This delay of 4–5 days was equivalent to the duration for which LH concentrations were significantly suppressed by 200 µg antagonist when administered to ovariectomized animals ( $N = 3$ ). Corpus luteum function during the cycle after GnRH antagonist treatment appeared normal according to the pattern of circulating progesterone. These results show that corpus luteum function and pre-ovulatory follicular development in the marmoset monkey are dependent on pituitary gonadotrophin secretion.

**Keywords:** GnRH antagonist; monkey; corpus luteum

## Introduction

The availability of potent selective antagonists of GnRH has provided new opportunities to investigate the role of pituitary LH in the control of corpus luteum function in primates. So far, however, the use of this approach has produced conflicting results. In an early study, Balmaceda *et al.* (1983) reported normal luteal function in rhesus monkeys after daily injections of GnRH antagonist throughout the luteal phase, thus suggesting that luteal function is independent of continued luteotrophic support. In contrast, convincing evidence that luteal function does depend upon pituitary LH secretion has been obtained by Fraser *et al.* (1985, 1986) who demonstrated that a single injection of GnRH antagonist during the mid-late luteal phase in stump-tailed monkeys results in inhibition of progesterone secretion and premature luteal regression. This discrepancy in findings is unlikely to be due to species differences since other experimental approaches resulting in pituitary

gonadotrophin deprivation in rhesus monkeys support the view that luteal function requires pituitary luteotrophic support (Hutchison & Zeleznik, 1984, 1985). A more likely explanation is that the results of Balmaceda *et al.* (1983) were due to incomplete suppression of LH release by an antagonist of lesser potency than that used in the later studies.

The use of GnRH antagonists to investigate the gonadotrophic requirements for corpus luteum function in other non-human primates has not been reported. In the present study we have examined the effects of a single subcutaneous injection of a novel highly potent GnRH antagonist on pituitary LH secretion and luteal function in the marmoset monkey, *Callithrix jacchus*. Since there is evidence in primates that luteal sensitivity to gonadotrophin deprivation changes with the age of the corpus luteum (Fraser *et al.*, 1985), the effects of acute withdrawal of LH were investigated during the early and mid-luteal phase. Luteal function was assessed by the measurement of circulating progesterone concentrations. The development of a sensitive microtitre plate enzyme immunoassay for the direct measurement of marmoset plasma progesterone is also described.

### Materials and Methods

**Animals.** Adult female marmoset monkeys weighing 350–400 g and having regular ovarian cycles were used. The animals were paired with castrated ( $N = 2$ ) or vasectomized ( $N = 6$ ) males and housed in the Institute of Zoology primate colony under conditions previously described (Harlow *et al.*, 1983; Hearn, 1983). Blood samples (0.2 ml) were collected from the femoral vein of unsedated animals into 1.0 ml heparinized syringes and placed immediately on ice. The blood was centrifuged at 500 g for 20 min and the plasma was stored in two aliquots at  $-20^{\circ}\text{C}$  until assayed.

Ovarian cycles were monitored throughout the study by the measurement of plasma progesterone concentrations. The day of ovulation was defined as that preceding the day on which progesterone concentrations rose above a value of 10 ng/ml and the last day of the luteal phase as the day preceding the fall of progesterone concentrations below 10 ng/ml (Harlow *et al.*, 1983). Marmosets in our colony have a mean  $\pm$  s.e.m. cycle length of  $28.6 \pm 1.0$  days with a luteal phase of  $19.2 \pm 0.6$  days (Harlow *et al.*, 1983).

**Experimental protocol.** Each animal was studied for one control cycle before receiving the GnRH antagonist [N-acetyl-D $\beta$ Nal1-D-pCl-Phe2-D-Phe3-D-Arg6-Phe7-Arg8-D-Ala10]NH<sub>2</sub> GnRH (Ipsen International, 75016 Paris, France). Luteal regression was induced during the mid-late luteal phase of control cycles by administration of a single intramuscular injection of 0.5  $\mu\text{g}$  cloprostenol (Estrumate: ICI, Macclesfield, U.K.), a PGF-2 $\alpha$  analogue. Treatment with cloprostenol in the marmoset causes immediate luteal regression followed by ovulation 9–12 days later (Summers *et al.*, 1985; Hodges *et al.*, 1987). This response to cloprostenol was utilized to provide a reference for evaluating any potential effects of GnRH antagonist treatment in terms of both corpus luteum function and subsequent follicular development.

During the subsequent luteal phase animals were injected with GnRH antagonist in 0.5 ml saline-propylene glycol (1:1, v/v), or with vehicle alone, according to one of the following experimental protocols: Group 1 ( $N = 4$ ), 100  $\mu\text{g}$  antagonist given on Day 10 or 11 of the luteal phase; Group 2 ( $N = 4$ ), 200  $\mu\text{g}$  antagonist given on Day 10 or 11 of the luteal phase; Group 3 ( $N = 4$ ), 200  $\mu\text{g}$  antagonist given on Day 5 or 6 of the luteal phase; Group 4 ( $N = 4$ ), vehicle alone given on Day 10 or 11 of the luteal phase (Day 1 of the luteal phase = first day after ovulation). Blood samples were collected every other day from cloprostenol treatment in control cycles until the end of the luteal phase of the cycle after GnRH antagonist treatment. Blood was collected 3 times per week after injection of vehicle. Progesterone was measured in all samples; LH was measured in samples collected on Days 0, 1, 2 and 4 after GnRH antagonist or vehicle treatment.

To observe the effects of the antagonist on LH secretion in more detail, 200  $\mu\text{g}$  antagonist were given to 3 ovariectomized females and blood samples were collected immediately before and 1, 2, 4, 8 and 12 h and 1, 2, 3, 5 and 7 days after the injection. LH was measured in all samples.

**Analysis of data.** Changes in LH concentrations in cyclic and ovariectomized animals were statistically evaluated using one-way analysis of variance followed by Duncan's multiple range test. The effects of antagonist treatment on subsequent follicular development were assessed by calculating the interval from the day of injection to the day on which progesterone values rose above 10 ng/ml. Data for treatment and respective control (cloprostenol-injected) cycles were compared using a paired *t* test. Since samples were collected every other day, data for each animal were plotted separately and the line connecting two adjacent sample points was used to estimate the day on which progesterone concentrations first exceeded a value of 10 ng/ml. Results obtained in this way agree well with those obtained from progesterone measurements in daily samples (Harlow *et al.*, 1983; J. K. Hodges, unpublished observations).

**Progesterone enzyme immunoassay.** Plasma progesterone was measured in unextracted samples using a modification of the heterologous enzyme immunoassay procedure described by Sauer *et al.* (1986). The antiserum was raised in a sheep after immunization against progesterone-11 $\alpha$ -hemisuccinate conjugated to ovalbumin. The gamma-globulin fraction

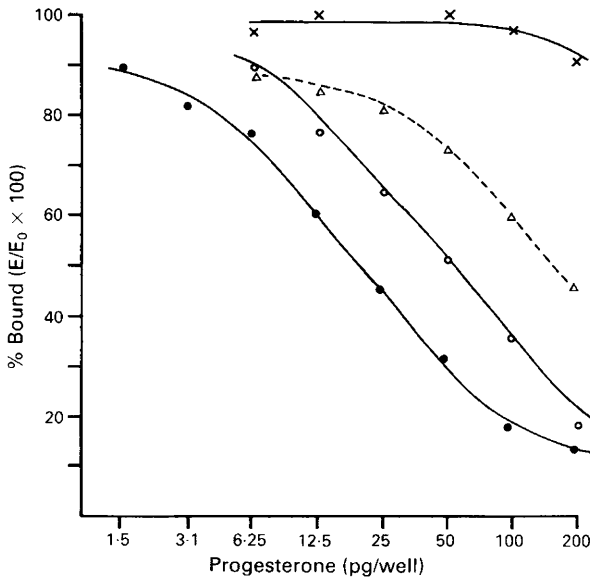


Fig. 1. Standard dose-response curve for the progesterone enzyme immunoassay (●) compared with serial dilutions of marmoset plasma from the follicular (△) and luteal (○) phase of the cycle and after removal of endogenous progesterone by treating with charcoal (×).

was precipitated with ammonium sulphate and absorbed free of ovalbumin antibodies before storage at  $-20^{\circ}\text{C}$ . Alkaline phosphatase (EC 3.1.3.1; type VII-T, from bovine interstitial mucosa; Sigma Chemical Co. Ltd, Poole, Dorset, U.K.) was conjugated to progesterone 11-glucuronide (gift from Dr J. E. T. Corrie, MRC Radioimmunoassay Team, Edinburgh) at a steroid:enzyme molar ratio of 2:1 using the active ester technique previously described in detail (Sauer *et al.*, 1986). The conjugate was stored in phosphate-azide-saline (PAS)-gelatin buffer (0.1 M, pH 7.0) at  $4^{\circ}\text{C}$  at which temperature it remains stable for at least 1 year.

The anti-progesterone gamma-globulin was diluted 1:2000 in 0.15 M-sodium acetate buffer, pH 5.0 and 200  $\mu\text{l}$  were added to each well of microtitre plates which were then covered and left for 3 h at room temperature in a covered plastic box lined with damp paper (humid chamber). The plates were emptied by inversion, PAS-gelatin buffer (300  $\mu\text{l}$ ) was added to each well and the plates were tightly sealed with plastic film before storing at  $4^{\circ}\text{C}$ .

**Assay procedure.** Microtitre plates coated with antibody were emptied, rinsed with PAS-gelatin buffer and blotted dry immediately before use. For each assay, 10  $\mu\text{l}$  of progesterone in toluene (1  $\mu\text{g}/\text{ml}$ ) were dried down under nitrogen and reconstituted in 1 ml standard diluent (PAS-gelatin buffer containing charcoal-treated marmoset plasma at 1:15, v/v). A 200  $\mu\text{l}$  sample of this solution was double diluted over the range 10 000–156 pg/ml (equivalent to 200–1.5 pg/well after further dilution with a solution of conjugate). Unextracted plasma samples (5  $\mu\text{l}$ ) and quality controls were diluted 1:30 with sample diluent (PAS-gelatin buffer containing charcoal-treated marmoset plasma at 1:30, v/v). Volumes of 100  $\mu\text{l}$  of diluted sample, quality control and standard were transferred to clean tubes to which 400  $\mu\text{l}$  of conjugate (1:1000) were added. The contents were mixed and 200  $\mu\text{l}$  volumes were added to duplicate wells to initiate the competition reaction. The plates were covered with a lid and incubated at room temperature ( $>20^{\circ}\text{C}$ ) for 3 h in a humid chamber. The plates were then emptied and washed three times with PAS-gelatin buffer (350  $\mu\text{l}/\text{well}$ ). Substrate [*p*-nitrophenyl phosphate, 15 mM in DEM buffer (pH 9.8), containing diethanolamine (1 M), MgCl (0.5 mM) and  $\text{NaN}_3$  (0.1%)] was added to each well (200  $\mu\text{l}$ ) and the plates were incubated for 1 h at  $37^{\circ}\text{C}$  in a humid chamber. The reaction was stopped by the addition of 50  $\mu\text{l}$  3 N-NaOH to each well. The absorbance (optical density) was measured at 405 nm in an automatic plate reader using a substrate-only well as a plate blank. Assay blanks were determined by addition of buffer and/or sample diluent (charcoal-treated marmoset plasma at 1:30, v/v) plus conjugate to duplicate wells; standard diluent plus conjugate was added to duplicate wells to determine enzyme activity bound to the antiserum in the absence of free progesterone (i.e.  $E_0$ ). A standard curve was constructed on a semi-log scale by plotting percentage bound ( $E/E_0 \times 100$ ) against amount of progesterone added ( $E$  and  $E_0$  are the absorbance readings in the presence and absence of progesterone respectively).

**Assay validation.** A typical EIA dose-response curve covering the range 1.5–200 pg progesterone is shown in Fig. 1. Under the assay conditions described, absorbance (optical density) readings at 405 nm were normally within the range 1.1–1.3 (0 pg progesterone) to 0.1–0.15 (200 pg progesterone). The sensitivity of the assay, defined as the amount of progesterone resulting in 90% binding compared to  $E_0$ , was 1.5–2.0 pg/well. At the high dilutions of marmoset plasma used, this gave a value for the limit of detection of the assay of 1.1–1.5 ng progesterone/ml.

Serial dilutions of marmoset plasma containing concentrations of progesterone typical of those found during the luteal and follicular phases of the cycle gave displacement curves parallel to that obtained with progesterone standards (Fig. 1). There was no significant displacement of binding with charcoal-treated marmoset plasma. Cross-reactivities of the antiserum with other steroids (tested at 50% E/E<sub>0</sub>) include: 11 $\alpha$ -hydroxyprogesterone, 52.9%; 5 $\beta$ -pregnane-3,20-dione, 36.3%; 5 $\alpha$ -pregnane-3,20-dione, 4.7%; other C21 steroids <2%; cortisol <0.01%; C19 and C18 steroids <0.1%.

Values for the intra-assay coefficient of variation, determined from repeated measurements of a high and low value marmoset plasma pool, were 5.3% at 23% E/E<sub>0</sub> (mean value 46.9 ng/ml,  $n = 40$ ) and 8.1% at 58% E/E<sub>0</sub> (mean value 11.8 ng/ml,  $n = 20$ ). The figures refer to within-plate variation. Corresponding values for the inter-assay coefficient of variation for high and low value plasma pools were 15.3% at 30% E/E<sub>0</sub> (mean value 46.5 ng/ml,  $n = 12$ ) and 14.5% at 59% E/E<sub>0</sub> (mean value 12.3 ng/ml,  $n = 12$ ).

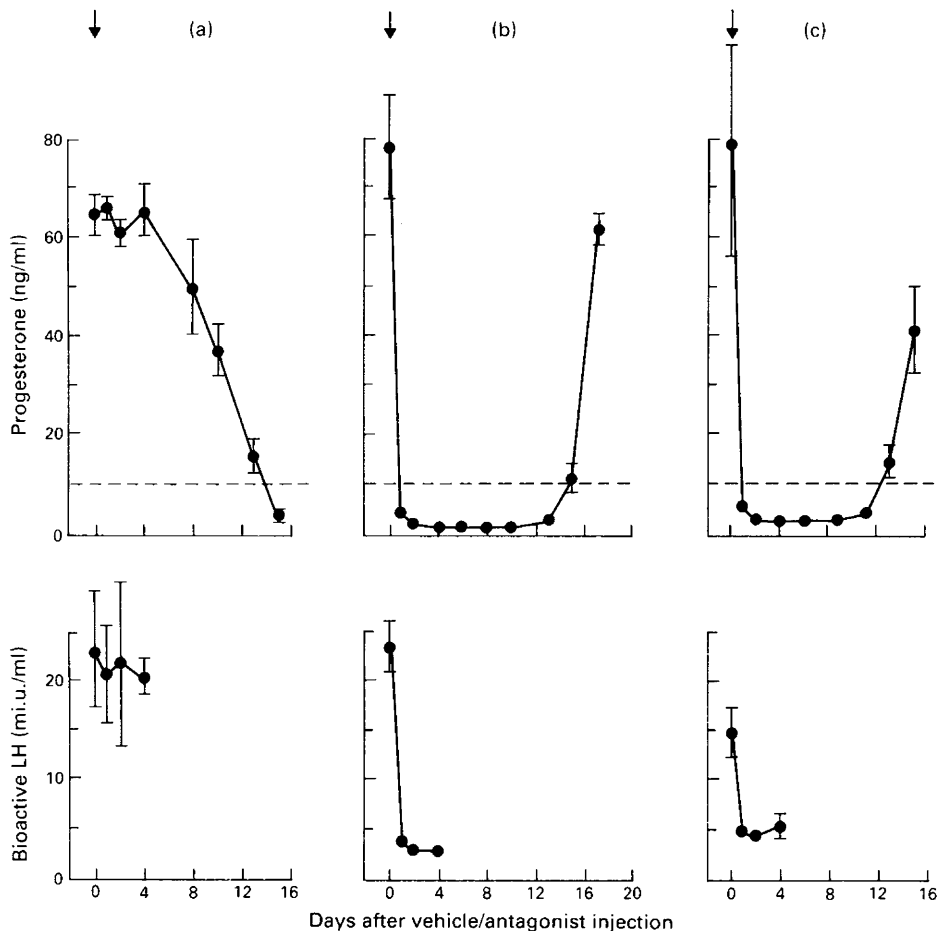
Accuracy was assessed in two separate assays by determining the recovery of known amounts (25–200  $\mu$ g) of progesterone standard added to marmoset plasma containing low levels of endogenous progesterone. Mean  $\pm$  s.d. values for recovery were  $97.1 \pm 5.4\%$  ( $n = 4$ ) and  $91.6 \pm 0.7\%$  ( $n = 4$ ). Progesterone values obtained using the enzyme immunoassay in a total of 252 plasma samples were compared with those from the same samples measured by a previously validated non-extraction radioimmunoassay (Summers *et al.*, 1985). The results showed a high degree of correlation between the two measurements over the range of values 1.5–95.0 ng/ml. The regression equation was  $y = 1.03x + 0.7$ , with a correlation coefficient ( $r$ ) of 0.94.

**LH bioassay.** Luteinizing hormone was measured using an in-vitro bioassay based on the production of testosterone by dispersed mouse Leydig cells (Van Damme *et al.*, 1974). Full details of the method and its validation for use in the marmoset have been described previously (Harlow *et al.*, 1984; Hodges *et al.*, 1987). Plasma samples were assayed in duplicate at two dilutions (1:40 and 1:80) and compared with the 2nd International Reference Preparation of human pituitary gonadotrophin (78/549) as LH standard. Testosterone production was measured by radioimmunoassay as described previously (Hodges *et al.*, 1987). The precision of the LH bioassay expressed as intra- and inter-assay coefficients of variation for replicate determinations of a marmoset plasma pool was <10% ( $n = 12$ ) and <15% ( $n = 6$ ).

## Results

The day of the luteal phase on which treatment was given was determined from the pattern of circulating progesterone concentrations (Day 1 of the luteal phase, i.e. one day after ovulation, was the day on which progesterone values rose above 10 ng/ml). Injection of vehicle on Day 10 or 11 of the luteal phase had no effect on the concentrations of progesterone or bioactive LH (Fig. 2). The mean  $\pm$  s.e.m. length of the luteal phase in animals receiving vehicle alone was  $21.3 \pm 1.8$  days ( $N = 3$ ) which is similar to that previously reported for animals in this colony ( $19.2 \pm 0.6$  days,  $N = 48$ ; Harlow *et al.*, 1983). In contrast, the injection of 200  $\mu$ g antagonist on Day 10 or 11 of the luteal phase caused a significant fall in plasma bioactive LH concentrations and a premature decline in luteal progesterone secretion. Mean  $\pm$  s.e.m. LH levels fell from  $23.4 \pm 3.2$  mi.u./ml immediately before injection to  $3.4 \pm 0.6$  mi.u./ml 24 h later ( $P < 0.01$ ). Concentrations remained significantly lower than pre-injection values until the end of the period of measurement on Day 4. In association with this decline in LH secretion there was a fall in progesterone concentrations from a mean  $\pm$  s.e.m. value of  $77.7 \pm 14.1$  ng/ml on the day of antagonist injection to  $3.6 \pm 0.5$  ng/ml 1 day later ( $P < 0.001$ ). Progesterone concentrations remained below 10 ng/ml (range 1.2–4.2 ng/ml) from 2 to 13 days after antagonist, rising to a mean value of  $11.1 \pm 2.9$  ng/ml on Day 15. In response to an injection of 100  $\mu$ g antagonist on Day 10 or 11 of the luteal phase, LH concentrations fell from a mean  $\pm$  s.e.m. value of  $14.4 \pm 1.3$  to  $4.7 \pm 0.8$  mi.u./ml within 1 day of treatment. As with the 200  $\mu$ g dose, mean LH concentrations 1, 2 and 4 days after injection were significantly lower than pre-injection values ( $P < 0.01$ ). Mean  $\pm$  s.e.m. progesterone concentrations declined significantly from  $77.9 \pm 24.3$  to  $5.2 \pm 1.7$  ng/ml after 1 day, remaining low or undetectable until Day 13 when they rose to a mean value of  $13.8 \pm 4.9$  ng/ml.

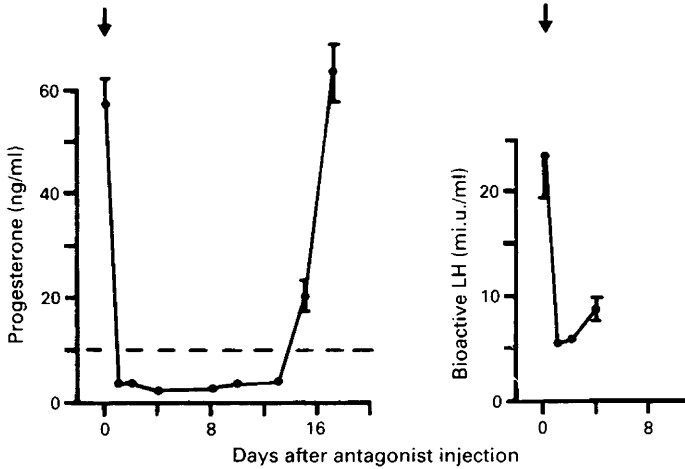
Injection of 200  $\mu$ g antagonist on Days 5 or 6 of the luteal phase also caused a fall in plasma bioactive LH concentrations and a premature decline in luteal progesterone secretion (Fig. 3). Mean LH concentrations on Days 1, 2 and 4 were all significantly lower than pre-injection values ( $P < 0.05$ – $P < 0.01$ ) although values on Day 4 were approximately double those measured in samples on Days 1 and 2. Progesterone concentrations fell to <10 ng/ml in all animals 1 day after injection and remained below this level until after Day 13. Mean  $\pm$  s.e.m. concentrations increased to  $20.1 \pm 2.2$  ng/ml 15 days after injection.



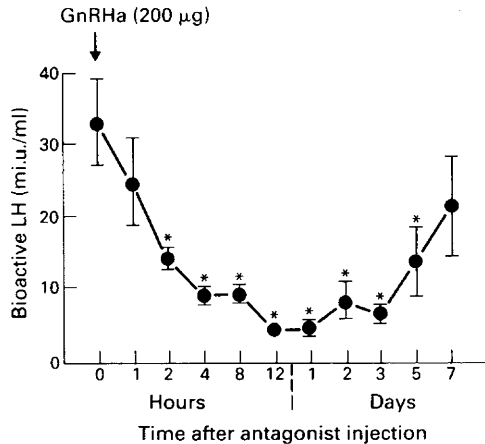
**Fig. 2.** Effect of (a) vehicle or (b) 200  $\mu$ g or (c) 100  $\mu$ g GnRH antagonist on plasma progesterone and bioactive LH concentrations in marmosets treated ( $\downarrow$ ) on Days 10/11 of the luteal phase. The pattern of progesterone secretion throughout the subsequent follicular phase and until after ovulation (day before the progesterone rise above 10 ng/ml) is shown for animals receiving antagonist. Values are mean  $\pm$  s.e.m. (where visible) for 4 animals/treatment.

The time course of the inhibition of LH secretion by 200  $\mu$ g antagonist was examined in detail in 3 ovariectomized animals. Mean LH concentrations fell significantly within 2 h of antagonist injection ( $P < 0.05$ ) and continued to decline to reach minimum values after 12–24 h (Fig. 4). LH concentrations remained low until Day 5 after which there was no significant difference from pre-injection values.

In intact animals the intervals from injection of antagonist to subsequent ovulation (i.e. day before the rise in plasma progesterone above 10 ng/ml) in the 3 treatment groups are shown in Table 1. In control groups the mean interval from cloprostenol-induced luteal regression to ovulation was approximately 9.8 days, which agrees with previously published data for animals in our colony (Summers *et al.*, 1985; Hodges *et al.*, 1987). Compared with this figure, the interval to ovulation was significantly lengthened in animals given 200  $\mu$ g antagonist (early and mid-luteal phase) ( $P < 0.01$ ) but not in animals given the 100  $\mu$ g dose. Moreover, there was no overlap in the range of values for control and antagonist-treated cycles (200  $\mu$ g dose).



**Fig. 3.** Effect of 200 µg GnRH antagonist on plasma progesterone and bioactive LH concentrations in marmosets treated (↓) on Days 5/6 of the luteal phase. The pattern of progesterone secretion throughout the subsequent follicular phase and until after ovulation is shown. Values are mean  $\pm$  s.e.m. (where visible), for 4 animals.



**Fig. 4.** Time course of suppression of plasma concentrations of bioactive LH after a single injection of GnRH antagonist (GnRHa) to ovariectomized marmosets. Values are mean  $\pm$  s.e.m. for 3 animals. \* $P < 0.01$  compared with preinjection (0 h) value.

Luteal function in the cycle after antagonist injection appeared normal as judged by the maximum concentrations of circulating progesterone attained (not shown) and by the length of time for which progesterone concentrations exceeded 10 ng/ml (i.e. length of the luteal phase). The mean lengths of the luteal phase in the 3 treatment groups (range 19.3–21.0 days) were therefore not significantly different from those in animals receiving vehicle alone ( $21.3 \pm 1.8$  days) or from those previously reported for animals in this colony ( $19.2 \pm 0.6$  days; Harlow *et al.*, 1983). A complete profile of progesterone concentrations during cloprostenol treatment, GnRH antagonist treatment (200 µg) and post-treatment cycles in an individual animal is shown in Fig. 5.

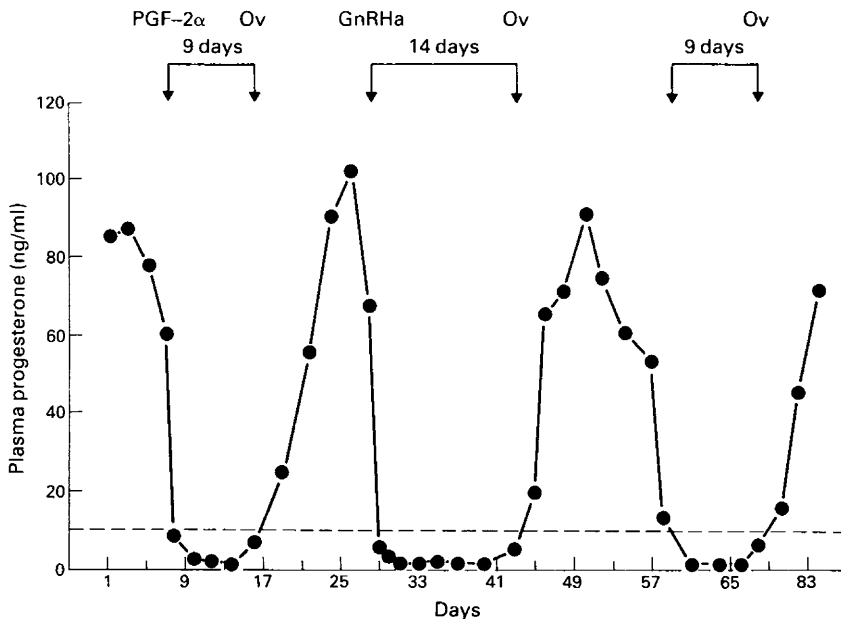
**Table 1.** Interval to ovulation in marmosets after induction of luteal regression with a single injection of cloprostenol or GnRH antagonist (GnRH<sub>a</sub>) during successive ovarian cycles

| Treatment    | Cycle 1        |                               | Cycle 2                                  |                |                               |
|--------------|----------------|-------------------------------|------------------------------------------|----------------|-------------------------------|
|              | No. of animals | Interval to ovulation (days)† | Treatment                                | No. of animals | Interval to ovulation (days)† |
| Cloprostenol | 4              | 9.5 ± 0.3 (8–10)              | GnRH <sub>a</sub> (200 µg),<br>Day 10/11 | 4              | *14.3 ± 0.4 (13–15)           |
| Cloprostenol | 4              | 9.8 ± 0.3 (9–10)              | GnRH <sub>a</sub> (100 µg),<br>Day 10/11 | 4              | 11.7 ± 0.8 (10–13)            |
| Cloprostenol | 4              | 9.8 ± 0.5 (9–11)              | GnRH <sub>a</sub> (200 µg),<br>Day 4/5   | 4              | *13.5 ± 0.3 (13–14)           |

Values are mean ± s.e.m. and range.

†Days from injection of cloprostenol or GnRH antagonist to the day before the rise in circulating progesterone concentrations above 10 ng/ml.

\*Significantly different from previous cycle.



**Fig. 5.** Complete progesterone profile in an individual marmoset, showing the interval to ovulation after cloprostenol (PGF-2 $\alpha$ ) and GnRH antagonist-induced luteal regression during successive ovarian cycles and after natural luteal regression in a third (post-treatment) cycle. Note the extended follicular-phase length after GnRH antagonist treatment and the subsequent normal pattern of luteal progesterone secretion. Ov = ovulation (day before the progesterone rise above 10 ng/ml).

## Discussion

The present study shows that a single subcutaneous injection of a GnRH antagonist between Days 5 and 11 of the luteal phase in the marmoset monkey causes a marked suppression of pituitary LH

secretion resulting in a rapid decline in circulating progesterone concentrations and luteal regression. The results provide evidence that in the marmoset progesterone secretion by the corpus luteum is dependent upon pituitary LH. Although some reports have suggested that primate corpus luteum function is independent of pituitary LH (Asch *et al.*, 1982; Balmaceda *et al.*, 1983) the consensus from more recent studies, including the present one, is that continued pituitary luteotrophic support is essential (Hutchison & Zeleznik, 1984, 1985; Fraser *et al.*, 1985, 1986).

Recent evidence also suggests that the relationship between corpus luteum function and pituitary LH varies with age, and that as the luteal phase progresses there is an increasing dependence upon luteotrophic support. Fraser *et al.* (1985, 1986) have reported that a single injection of GnRH antagonist into stump-tailed macaques caused sustained suppression of progesterone secretion (i.e. luteal regression) when given during the mid-late luteal phase, whereas similar treatment during the early luteal phase (Day 7 or before) resulted in only temporary reduction in progesterone secretion and an apparent recovery of normal luteal function. In the marmoset, GnRH antagonist treatment was equally effective in inducing luteal regression when given during the early or mid-luteal phase. Whilst it is possible that species differences exist in the ability of the early corpus luteum to recover from acute LH deprivation, a more likely explanation for this difference is that the dose and/or potency of the antagonist used in the marmoset resulted in a more effective and/or sustained suppression of LH secretion than that achieved in the macaque studies. This is supported by the data from ovariectomized marmosets (Fig. 4) indicating that treatment with 200 µg GnRH antagonist results in suppression of LH for at least 5 days. Although the LH response to GnRH antagonist in intact and gonadectomized marmosets may not be directly comparable, the results nevertheless suggest that a similar dose of antagonist given on Day 5 or 6 of the luteal phase would be expected to suppress LH secretion until at least Day 10 or 11. This represents a more prolonged period of LH deprivation than achieved in the macaque by Fraser *et al.* (1985, 1986) and probably one from which the corpus luteum (of any primate species) would be unlikely to recover. Fraser *et al.* (1987) have, in fact, now demonstrated that longer term treatment with GnRH antagonist (3 consecutive daily injections) during the early luteal phase in the macaque does result in luteal regression.

Although the primary mechanism of action of the GnRH antagonist is to block GnRH receptors on the pituitary gonadotroph, specific binding of GnRH and its analogues to luteal tissue has been demonstrated for various species including primates (Bramley *et al.*, 1985). The possibility of a direct action of the GnRH antagonist on primate ovarian cells therefore cannot be excluded. However, in view of the suppression of LH secretion observed it is reasonable to assume that the GnRH antagonist-mediated effects on progesterone secretion in the present study were principally due to an action at the pituitary level.

In addition to the effects on corpus luteum function, treatment with the 200 µg dose of GnRH antagonist resulted in a prolongation of the subsequent follicular phase and a delay in the time to ovulation. Thus, the interval from GnRH antagonist-induced luteal regression to the rise in progesterone concentrations above 10 ng/ml (i.e. day after ovulation) was on average 5 days longer than the comparable interval following induction of luteal regression with cloprostenol (Table 1; Summers *et al.*, 1985; Hodges *et al.*, 1987). Since this prolongation approximates the duration for which LH values were suppressed in ovariectomized females, it is reasonable to assume that the delay in ovulation resulted from a temporary arrest of follicular development due to inadequate levels of circulating gonadotrophins for several days after GnRH antagonist treatment. Although measurement of LH and/or FSH concentrations following cloprostenol and GnRH antagonist-induced luteal regression are needed to confirm this, the present results nevertheless provide indirect evidence that preovulatory follicular development in the marmoset is sensitive to transient withdrawal of pituitary gonadotrophin support.

Delays in ovulation after temporary suppression of gonadotrophin secretion by GnRH antagonists have also been reported for rhesus monkeys (Balmaceda *et al.*, 1981; Borghi *et al.*, 1983) and women (Mais *et al.*, 1986). In these studies, in which repeated daily injections of antagonist were



given for 3–6 days from the beginning of the follicular phase, the delay in ovulation was generally equivalent to the duration for which the antagonist was administered. The results for the marmoset are therefore impressive in that a substantial delay in ovulation (up to 5 days) was achieved with only a single injection of antagonist (200 µg). Although comparison of effects between the various studies is difficult due to the use of different antagonists at different doses relative to body weight, the present results clearly indicate that the antagonist used in this study is a highly potent agent in the suppression of primate gonadotrophin secretion.

Despite the disruption of preovulatory follicular development after GnRH antagonist treatment, subsequent ovulation and corpus luteum function appeared normal as judged by the pattern and concentrations of circulating progesterone (see Fig. 5). Similar findings of normal luteal function have also been reported for other primate species following the use of agonistic (Skarin *et al.*, 1982; Borghi *et al.*, 1983) and antagonistic (Asch *et al.*, 1984; Mais *et al.*, 1986) analogues of GnRH to suppress follicular development. The single observation by Sheehan *et al.* (1982) of inadequate luteal phases following early follicular phase administration of a GnRH antagonist to women therefore remains to be substantiated.

In conclusion, this study shows that luteal progesterone secretion in the marmoset monkey is largely dependent upon pituitary LH support, thus providing further evidence for the essential role of LH in the maintenance of primate corpus luteum function. The results also demonstrate that preovulatory follicular development is sensitive to transient withdrawal of pituitary LH and/or FSH during the early follicular phase with the result that ovulation is delayed. The use of GnRH antagonists as probes provides excellent opportunities to elucidate the gonadotrophic control of follicular development and corpus luteum function in primates.

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