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Composite Pattern of Circulating LH, FSH, Estradiol, and Progesterone during the Menstrual Cycle in Cynomolgus Monkeys (39834)

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Traditionally, the rhesus monkey (*Macaca mulatta*) has been used widely as the nonhuman primate of choice for investigating factors that regulate pituitary and ovarian function not only during the normal menstrual cycle (1, 2), but also during pregnancy (3, 4), and now, menopause (5) as well. Recently, the availability of rhesus monkeys for such research has declined; restrictions imposed on the importation of rhesus monkeys have limited the supply and have increased the cost markedly.

The potential of cynomolgus monkeys (*Macaca fascicularis*) as an alternative primate model suitable for similar kinds of investigations is clearly indicated by earlier studies (6–8). Yet, among published reports of circulating hormone levels during the menstrual cycle of this macaque (9–11), none provides a composite description of temporal relationships among circulating gonadotropins and ovarian steroids. Notably, no description of the patterns of follicle stimulating hormone (FSH) secretion has been reported for this species. As a prelude to contemplated studies that require thorough baseline data, circulating levels of FSH, luteinizing hormone (LH), estradiol-17β, and progesterone were measured in daily serum samples throughout the menstrual cycles of adult cynomolgus monkeys.

Materials and methods. The housing, inspection for menstruation, and collection of blood samples of the seven regularly cycling adult cynomolgus monkeys used in this study were conducted according to procedures outlined previously for our rhesus monkey colony (12, 13). Femoral blood samples (3.0–3.5 ml) were collected each morning (0900–1100 hr) from the onset of menses (Day 1) until the next menstrual flow. Serum was harvested after centrifugation and was stored at $-10°$ until assayed. Each sample was assayed for both pituitary gonadotropins and estradiol and progesterone.

Standard radioimmunoassay procedures with slight modifications were used to measure LH (14), FSH (15), progesterone (16), and estradiol-17β (17). Serum from an ovariectomized cynomolgus monkey produced responses parallel to those of a rhesus monkey standard (LER-1909-2, LH:0.003 × NIH-LH-S1, FSH:0.05 × NIH-FSH-S1) used in both gonadotropin assays. The estradiol antiserum employed was generously provided by Dr. K. Wright and Dr. D. C. Collins, Emory University, Atlanta, Georgia. This antiserum (E_2TG-K), produced in a rabbit immunized with 17β-estradiol-6-(*O*-carboxymethyl) oxime–bovine thyroglobulin, was used at an initial dilution of 1:64,000, which bound about 50% of the tritiated steroid ([2,4,6,7-^{3}H]estradiol-17β, New England Nuclear, 100 Ci/mmole; 10 pg/tube). Estrone cross reaction was 1.2% and, for estriol, 0.47%. Points in the standard curve ranged from 2 to 100 pg. Benzene extracts of the gelatin buffer diluent and ovariectomized monkey serum produced responses no different from the zero-dose control. Recovery of authentic steroid added to ovariectomized monkey serum averaged 70–80%. Serum samples were assayed without chromatography. The corrected relative sensitivity was about 12–15 pg/ml. All samples from an animal were run in a single assay to obviate the contribution

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of between-assay variation.

Results and Discussion. As depicted in Fig. 1, the composite hormonal pattern during the menstrual cycle in cynomolgus monkeys is similar to that of rhesus monkeys (1) and women (18). Coincident midcycle elevations of LH and FSH lasting a single day occurred at a mean interval of 11.4 ± 1.1 days ($\bar{X}$ ± SE) after the onset of menstrual flow; the ensuing luteal phase averaged 15.7 ± 1.2 days. In all monkeys, the midcycle surge of gonadotropins was preceded by a progressive increase in estradiol levels, so that, in five of seven animals, the estrogen zenith was reached one day before the gonadotropin peak. In the other two monkeys, the peaks in circulating estradiol and gonadotropins were coincident. After the midcycle gonadotropin surges, progesterone levels followed a monophasic pattern, reaching maximal concentrations during the midluteal phase.

Despite clear qualitative similarities, small, but distinct, quantitative differences are evident between gonadotropin levels in cynomolgus and rhesus monkeys. When compared against the same standard or when corrected for different standards, average tonic and surge levels of LH were generally lower in cynomolgus monkeys. FSH levels also tended to be lower than those of rhesus monkeys. Even though the values depicted here were measured in sera collected during summer months, the apparent species difference is probably not seasonal, since gonadotropin levels in another group of cynomolgus monkeys sampled during the fall and winter were also lower than typical levels in rhesus monkeys (Goodman and Hodgen, unpublished).

Circulating levels of estradiol and progesterone, on the other hand, were indistinguishable from those reported for rhesus monkeys (1, 15). The use of highly specific antisera for the quantitation of estradiol and progesterone in this study may explain the fourfold higher estimates of plasma "estrogens" and "progestins" previously reported for this species (10).

That the composite hormonal pattern illustrated is representative of normal ovulatory menstrual cycles in this macaque is indicated by presumptive indicators of ovulation, namely, a phase of elevated progesterone secretion after the LH surge. This inference is supported by two additional findings: (i) During the very next cycle, six of seven monkeys studied here became pregnant after cohabitation with a male on Days 11 to 13; and (ii) fresh corpora lutea were observed on Days 16 to 19 in another group of cynomolgus monkeys having very similar hormonal patterns (Goodman and Hodgen, unpublished).

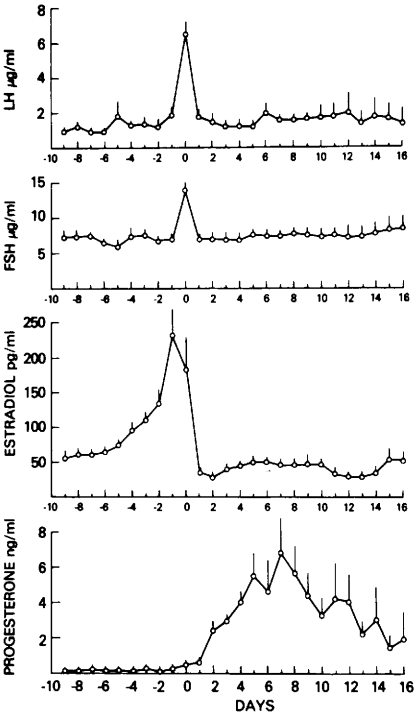

FIG. 1. Composite pattern of circulating levels of LH, FSH, estradiol, and progesterone throughout the menstrual cycle in seven cynomolgus monkeys. LH and FSH levels are expressed in terms of rhesus monkey pituitary reference preparation LER 1909-2 (FSH: 0.05×NIH-FSH-S1; LH: 0.003×NIH-LH-S1). Vertical bars at each point indicate 1 SEM.

Several factors have been considered in the choice of the cynomolgus monkey as an alternative model for primate reproductive studies. First, general information on reproductive patterns in this species is already available (6, 7, 8). Husbandry practices and surgical procedures currently employed for rhesus monkeys have been found to be satisfactory for this macaque, as well. In addition, systematic laparoscopic observations

of follicle growth (19) and the composite endocrine pattern described here indicate the close similarity of these reproductive parameters in rhesus and cynomolgus monkeys. Thus, future studies of primate reproductive physiology will likely exploit these macaques on a larger scale.

Summary. Circulating levels of LH, FSH, estradiol and progesterone were measured by radioimmunoassays in daily serum samples throughout the menstrual cycle of seven adult cynomolgus monkeys. The composite pattern of these hormones in cynomolgus monkeys strongly resembles that reported for rhesus monkeys, and is similar to that in the menstrual cycle of women. Thus, in the face of diminishing supplies and higher costs of rhesus monkeys, the cynomolgus monkey can also serve effectively as a model for studies in primate reproduction.

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