INDUCTION AND TIMING OF SINGLE AND MULTIPLE OVULATIONS IN THE SQUIRREL MONKEY (*SAIMIRI SCIUREUS*)

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Summary. Fifty-six squirrel monkeys were studied to develop means of accurate ovulation induction. Ovaries were exposed to determine ovulation time. Pre-treatment with progesterone to suppress spontaneous ovulation (5 mg daily for 5 days) was followed by: (a) no FSH, (b) 1 mg FSH, (c) human menopausal gonadotrophin (HMG), equivalent to 75 i.u. FSH, or (d) 200 i.u. pregnant mare's serum gonadotrophin (PMSG), each for 4 additional days. Ovulation was induced in 43% of the animals with either 250 or 500 i.u. of human chorionic gonadotrophin (HCG) administered intravenously (i.v.) or intramuscularly (i.m.). Four double ovulations were observed, the others were single ovulations. No follicular development occurred in animals which did not receive an exogenous source of FSH. FSH was superior to HMG and PMSG in its ability to promote follicular growth. Nearly twice as many animals ovulated after 500 i.u. HCG than with 250 i.u. Injection of the HCG i.v. or i.m. did not affect the percentage of animals that ovulated but greater variation in the time of ovulation was noted with i.v. injections.

INTRODUCTION

Accurate timing of ovulation is a prerequisite for definitive studies of the early stages of fertilization in any species. The period during which the ovum can be fertilized, limited to a few hours in most species, is of critical importance for studies on sperm capacitation and *in vitro* fertilization (Dukelow, Chernoff & Williams, 1967). In view of this fact and of the importance of the non-human primate in biomedical research on reproduction and fertility control, we have developed techniques for inducing single or multiple ovulations at a predictable time in the squirrel monkey.

Ovulation has been induced in other non-human primates, chiefly in *Macaca mulatta*. As early as 1935, Hisaw, Greep & Fevold, using crude ovine anterior pituitary extracts, reported ovulation induction in three out of four macaques. Hartman (1938) reported a total of seven ovulations out of 104

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cycles in anovulatory adult rhesus monkeys. In 1942, he reported an additional six ovulations out of forty-six cycles. Pfeiffer (1950) attempted to prevent ovulation in rhesus monkeys with 0.5 mg of progesterone daily from the 10th to the 14th day of the cycle. Four ovulations were obtained from eleven monkeys after the end of treatment.

Most of the work on the induction of ovulation in non-human primates has been carried out by van Wagenen and her colleagues and has been recently summarized (van Wagenen, 1968). Beginning in 1935, she unsuccessfully attempted to induce ovulation in macaques with pregnant mare's serum gonadotrophin (PMSG) (van Wagenen & Cole, 1938). Later work with purified FSH and interstitial cell stimulating hormone (ICSH) of ovine origin was ineffective in inducing ovulation. In 1956, Knobil, Morse & Greep reported the importance of species-specificity in some primate pituitary hormones. This led to the important observation (van Wagenen & Simpson, 1957a, b; Simpson & van Wagenen, 1957) that in a significant number of cases, multiple ovulation was obtained with gonadotrophins of primate origin. Subsequently, Knobil, Kostyo & Greep (1959) induced ovulation in hypophysectomized macaques by treatment with porcine FSH and human chorionic gonadotrophin (HCG).

In the intervening years, various ovulation-inducing agents have been studied for their effect on ovulation in rhesus monkeys. Dede & Plentl (1966) used Pergonal (human menopausal gonadotrophin, HMG) injections for 8 to 10 days followed by 2-day injections of HMG and HCG. The animals were then mated or artificially inseminated and pregnancies were obtained.

Wan & Balin (1969), using HMG/HCG, clomiphene citrate and DL-18-methyl oestril to induce ovulation in macaques, were successful in 60%, 59%, and 32% of the treated cycles, respectively. They were also successful in obtaining high incidences of single ovulations in contrast to Simpson & van Wagenen (1962), who reported multiple ovulations on each ovary.

Bennett (1967a, b) induced multiple ovulation in the squirrel monkey with various regimens of PMSG and HCG. He demonstrated that ova can be recovered from the oviducts and suggested that, probably because of the elevated level of oestrogen from the ovary, tubal transport could be speeded up by the high level of PMSG employed. Bennett began injections without reference to the stage of the cycle. The duration of the oestrous cycle in *Saimiri* is reported to range from 6.0 to 9.5 days by Rosenblum, Nathan, Nelson & Kaufman (1967) and from 23 to 69 days by Denniston (1964).

So far, no attempt appears to have been made to induce ovulation with the specific objective of timing ovulation accurately and of recovering the ovum within the period during which it can be fertilized.

MATERIALS AND METHOD

Forty-six mature squirrel monkeys (*Saimiri sciureus*) of the Brazilian and Peruvian types (Hill, 1960) were housed in wire cages, two to three animals per cage. All monkeys were injected intramuscularly (i.m.) with 5 mg of progesterone dissolved in 0.25 ml of cottonseed oil daily for 5 days to suppress spontaneous ovulation. After a 4-day lapse following progesterone treatment,

six animals were injected intravenously (i.v.) with either 250 or 500 i.u. of HCG to induce ovulation in order to ascertain whether this treatment alone would induce follicular growth and ovulation. The ovaries were exposed by a midline incision 10 to 12 hr later and examined for evidence of follicular growth and ovulation.

Three sources of FSH were studied in order to determine their potency for initiating follicular growth. (1) FSH-P (Armour-Baldwin Laboratories), a lyophilized product obtained from the pituitaries of domestic animals, was administered subcutaneously (s.c.) daily for 4 days after progesterone pre-treatment. (2) HMG (Ortho Research Foundation, Lot no. 2282) containing 75 i.u. of FSH and 25 i.u. LH was injected s.c. for either 2 or 4 days after cessation of the progesterone pre-treatment. (3) PMSG (Gonadogen, Upjohn Company) was injected daily in doses of 200 i.u. for 4 days.

TABLE 1
EFFECT OF VARIOUS SOURCES OF FSH ON OVULATION

<i>Treatment</i>	<i>No. of animals</i>	<i>Mean no. ovulation points/animal</i>	<i>% animals ovulating</i>	<i>Mean no. of ripe follicles/animal</i>
Progesterone only, no FSH source	6	0	0	0
Progesterone, FSH-P	23	1.3	56.6	—
Progesterone, HMG	5	1.0	20.0	5.3
Progesterone, PMSG	12	1.0	25.0	1.7

Only HCG was used to induce ovulation. This was administered at a level of either 250 or 500 i.u. in physiological saline, either i.v. or i.m. After midline exposure of the ovaries, the animals were kept under anaesthesia for 4 hr. At hourly intervals, the ovaries were examined and photographed to ascertain the time of ovulation.

In an effort to duplicate the superovulation obtained by Bennett (1967a), a 9-day course of injections was administered to four animals without the progesterone pre-treatment: 200 i.u. of PMSG were given for 5 days, followed by 4 days of PMSG and 500 i.u. of HCG. The ovaries were examined on Day 11 for ovulation and the oviducts were removed and flushed for ovum recovery. To determine if ovulation occurred after the surgical procedure, ten additional animals were subjected to the progesterone-FSH-P-HCG (500 i.u.) procedure and examined 40 hr after the last injection.

Parts of this work relating to FSH-induced ovulation have been published in a preliminary report (Dukelow, Guilloud, Willcox & Cline, 1969).

RESULTS

Attempts at inducing follicular growth and ovulation following pre-treatment with progesterone were unsuccessful (Table 1); no follicular development was noted in any of the six animals and the ovaries were quiescent (Pl. 1, Fig. 1).

Out of a total of forty observations where an exogenous source of FSH was used, seventeen (42.5%) animals ovulated with a total of twenty-one ovulation

points. Single ovulations were noted in 76.4% and double ovulations in 23.6%. No higher rate of ovulation occurred except in those animals superovulated by the Bennett technique. FSH-P was the most effective treatment in terms of ovulation points per animal and in the percentage of ovulations in response to HCG injection. HMG stimulated a high incidence of follicular development (mean of 5.3 follicles per animal), but the incidence of ovulation was only 20%. Similarly, PMSG administered once daily for 4 days did not stimulate a high incidence of follicular growth, and only 25% of these animals ovulated.

Five hundred i.u. of HCG were superior to 250 i.u. in inducing ovulation but the percentage of ovulation from i.m. and i.v. administration did not differ

TABLE 2
THE EFFECT OF THE AMOUNT AND TYPE OF HCG INJECTION ON OVULATION

<i>Treatment</i>	<i>No. of animals</i>	<i>Mean no. of ovulation points</i>	<i>% animals ovulating</i>	<i>Time range after HCG (hr:min)</i>	<i>Minimum time between HCG and ovulation (hr:min)</i>
250 i.u.	15	1.25	26.7	< 6:31 to 11:25	4:54
500 i.u.	25	1.23	52.1	< 7:47 to 11:54	3:07
i.m. injection	10	1.00	40.0	10:13 to 11:54	1:40
i.v. injection	30	1.31	43.4	< 6:31 to 11:40	5:09

TABLE 3
TIMED OVULATIONS IN
SAIMIRI (HR:MIN POST-
HCG)

<i>Type of injection</i>	
<i>i.v.</i>	<i>i.m.</i>
11:40	11:54
6:57	11:09
7:10	10:13
10:53	11:25
8:34	
8:20	

significantly (Table 2). The interaction between the route of injection and amount of HCG was not statistically significant and, therefore, the data for route and dose are pooled separately in Table 2. The ovulation points were distinct, red protruberances on the surface of the ovary (Pl. 1, Fig. 2).

Ovulation occurred during the surgical procedure in ten animals and the time from HCG injection to ovulation was calculated (Table 3). For three of them, this was a second ovulation, the first having occurred before surgery. Of the seventeen monkeys ovulating, ten ovulated before surgery, some earlier than 6 hr 32 min after HCG injections. All of these early ovulations occurred in animals receiving an i.v. injection of HCG. The time of ovulation in animals

PLATE 1

FIG. 1. Quiescent ovaries of *Saimiri* treated with progesterone alone (no FSH source).

FIG. 2. Two fresh ovulation points on the left ovary of *Saimiri*.

(Facing p. 306)

receiving an i.m. injection of HCG ranged from 10 hr 13 min to 11 hr 54 min. The greatest variability in ovulation time occurred with i.v. injections, with a minimum variability of 5 hr 9 min compared to 1 hr 40 min with i.m. injection. In four animals, double ovulation was observed (all with HCG administered intravenously) and the minimum difference in time between first and second ovulation was calculated (Table 4). In all cases, the first ovulation occurred before surgery. The highest minimum difference between first and second ovulations was 2 hr 38 min.

TABLE 4
TIME OF DOUBLE OVULATIONS

<i>Time (hr:min)</i>	<i>Minimum difference between ovulations (hr:min)</i>
Both < 11:16	?
< 9:05 and 11:40	2:35
< 6:31 and 6:57	0:26
< 8:42 and 10:53	2:38

All i.v. injections.

TABLE 5
OVULATION DIFFERENCES BETWEEN SAIMIRI OF BRAZILIAN OR PERUVIAN ORIGIN

<i>Saimiri type</i>	<i>No. of animals</i>	<i>Mean no. of ovulation points</i>	<i>% animals ovulating</i>	<i>Time range after HCG (hr:min)</i>	<i>Minimum time between HCG and ovulation (hr:min)</i>
Brazilian	28	1.23	46.4	< 6:31 to 11:54	5:23
Peruvian	12	1.25	33.3	< 8:05 to 10:53	2:48

The mean number of ovulation points, the percentage of animals ovulating, and the range in time of ovulation did not differ significantly in Brazilian- and Peruvian-type squirrel monkeys (Table 5).

When the superovulation technique of Bennett (5 days PMSG, 4 days PMSG-HCG) was performed on four animals, a total of nineteen ovulation points ranging from two to seven (or an average of 4.75 per animal) was observed. A few ova were recovered by flushing the oviduct but because of technical problems, the number of ova recovered is not considered relevant to this study.

Of the ten animals observed for ovulation 40 hr after HCG, six had ovulated with an average of 1.9 ovulation points per animal, indicating that while ova recovery at 11 to 12 hr after HCG will yield freshly ovulated ova, ovulation can occur later.

DISCUSSION

The squirrel monkey, now widely used in the research laboratory because of size, cost, and adaptability to surgical procedures, ranks second only to the rhesus in popularity. The successful induction of consistent ovulation in this species has proved the squirrel monkey to be suitable for studies on the period

of progestation, artificial inhibition of ovulation, and on primate sperm capacitation (Dukelow & Chernoff, 1969) and *in vitro* fertilization.

The present study demonstrates that a reasonable level of ovulation (56%) can be attained in the squirrel monkey with a regimen of 5 days of progesterone followed by 4 days of FSH and an injection of 500 i.u. of HCG. HMG caused an adequate number of follicles per animal to develop but ovulation was induced only in relatively few animals. Similarly PMSG, administered only once daily for 4 days, was not sufficient to cause large numbers of follicles to develop. If numerous follicles and superovulation are desired, PMSG must be administered twice daily over a 9-day period and combined with 250 to 500 i.u. of HCG during the last 4 days. Under this regimen, superovulation occurred and thus confirmed the work of Bennett (1967a) who obtained an average of 5.3 ovulation points per ovary in two animals.

As one would expect, ovulation occurs more rapidly with i.v. injection of HCG; moreover, the variability of the time of ovulation is much greater. For the accurate timing of ovulation and the higher probability of obtaining a freshly ovulated ovum (1 to 2 hr old), i.m. injections should be used followed by surgical recovery 11 to 12 hr later.

The above regimen will yield single ovulations in about 75% of the cases, with double ovulations occurring in the rest. Multiple ovulations are of greater importance to experimental researchers than to observers of the animal in the wild state since twinning is quite rare in this species (Napier & Napier, 1967). The interesting variation in time between ovulations (up to 2 hr 38 min) corresponds to the report of Harper (1961) who demonstrated that ovulation can be spread over a period of nearly 4 hr in the rabbit.

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