REPRODUCTIVE BIOLOGY OF MACACA FASCICULARIS

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ABSTRACT

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MACACA FASCICULARIS

By

Dennis Allen Jewett

The present studies were undertaken in order to characterize basic reproductive parameters in *Macaca fascicularis*, to develop a system of follicular puncture and aspiration with the laparoscope, to adapt photographic techniques to the laparoscope, and to identify ovarian follicular morphology near ovulation.

Menstrual cycle records were kept on all of the females in the colony for a period of 8 months. The mean cycle length for 129 cycles was 30.8 days; the median, 33 days; and the mode, 28 days. Cycles ranging from 27 to 32 days in length comprised 66.4% of all cycles. Regular cycles were scored on the basis of menses beginning within 2 days of a projected date based on an average of 6 previous cycles. Regular cycles were observed in 62.4% of the cases. The mean cycle length for regular cycles was 29.2 days. Forty-five percent of all females had regular cycles at least 70% of the time. Estimating ovulation time by laparoscopy, the mean length of the follicular phase was 15.4 ± 3.0 days; the luteal phase 13.6 ± 2.5 days. The duration of

menstrual flow for 150 cycles averaged 2.8 days. Menses ranged from 1 to 8 days in length, with 90.7% of the cases ranging from 1 to 4 days in length. For a given duration of menses, the percentage of cases of light flow increased as the end of the menstrual period was reached.

As a result of 31 mating sessions, each of 30 minutes duration, 4 pregnancies were achieved. Average cycle lengths varied from 28 to 32 days, and all pregnancies occurred between 16 and 18 days before the next expected menstruation. The mean gestation length was 164.5 days, with implantation bleeding beginning on day 19 of pregnancy and lasting for 7.3 days.

A PMS/HCG regime was introduced to attempt superovulation in *M. fascicularis*. Several characteristic follicles resulted from this regime. A device for puncture and aspiration of ovarian follicles was developed and used to evacuate the follicles in the above regime. A total of 1.5 ml of follicular fluid was collected.

Rectal temperature recordings were made of monkeys which had been removed from their cages and manually restrained. The recordings exhibited fluctuations which precluded the detection of thermal shifts which might be associated with ovulation.

Mating behavior in *M. fascicularis* was quantified as a basis for a scoring system intended to provide a means of selecting animals for breeding studies. The female Reproduction Performance Index and Success Ratio were

observed to provide the most reliable index of mating behavior.

Several combinations of cameras, films, and settings were used to develop a photography system for use with the laparoscope. For standard color photography, the Olympus Pen-F, and Canon TL, with Ektachrome EHB-135 type film, produced the best result. For infrared photography, Ektachrome infrared film, in conjunction with a specific combination of filters, was used to clarify the vasculature associated with follicular development, and to diagnose early developing follicles.

The identification of the morphology of follicular maturation was pursued in *M. fascicularis* in order to facilitate ovulation detection and induction studies and the aspiration of mature ova. A vascular network emerging on the surface of the ovary 30 hours before ovulation provided the most reliable indication of follicular development. Within 10 hours of ovulation a single vessel became established on the center of the follicular wall. Stigmata were observed on either side of this vessel. Post-ovulatory follicular changes were found to permit the diagnosis of ovulation by laparoscopy.

REPRODUCTIVE BIOLOGY OF

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A THESIS

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TO THE MEMORY OF MY BROTHER

MARK JEWETT

But there's no vocabulary
For love within a family, love that's lived in
But not looked at, love within the light of which
All else is seen, the love within which
All other love finds speech.
This love is silent.

--T. S. Eliot, The Elder Statesman

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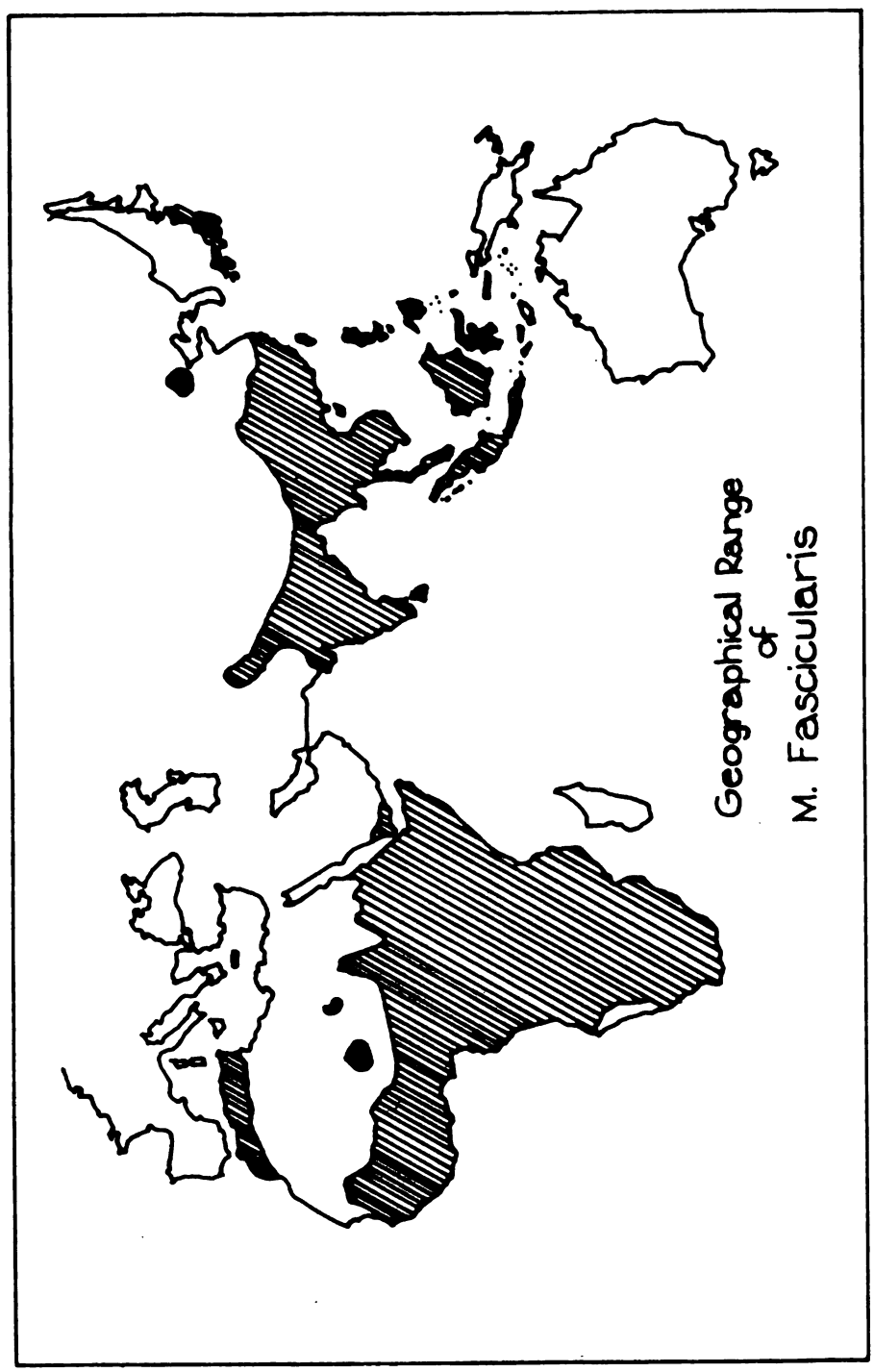
INTRODUCTION

The use of nonhuman primates in research has gained considerable popularity in recent years due to the close anatomical and physiological correlation to their human counterparts. Much of the attention has been directed toward the rhesus monkey (*Macaca mulatta*), but other species of macaques are now taking their places in the literature.

Macaca fascicularis is an example of a macaque which is very similar in many respects to *M. mulatta*, but about which little is presently found in the literature. *M. fascicularis* has been given the following common names: the Irus monkey, Java monkey, Crab-eating macaque, the Malayan monkey, and the Cynomolgus macaque. (For further discussion of taxonomy, see Appendix I). The geographic range of this Old World species includes North Africa, Gibraltar, Asia from East Afghanistan and Tibet to China, Japan, and Formosa, south to India and Ceylon, throughout Southeast Asia including Sumatra, Java, Borneo, the Philippines, and Celebes, and many offshore islands (Figure 1). Macaques are partly arboreal and partly terrestrial, usually sleeping in trees to avoid predators; but also frequenting cliffs and rocky places. *M. fascicularis* has been found most often near the coast in tidal creeks and mangrove

FIGURE 1



swamps. This species is grey to pale-yellowish brown with crown hairs directed backwards, sometimes forming a crest on the midline. The adults have cheek hairs which form a fringe of whiskers on and about the face. The tail is equal to the head and body length or slightly longer. The weight range of the average male is 3.5 to 8.3 Kg; the female, 2.5 to 5.7 Kg (Napier and Napier, 1967). The hand is prehensile with the thumb fully opposable. The males reach sexual maturity at four to five years; females, three to four years; and full growth is not complete until the sixth year. Longevity of *M. fascicularis* is reported to average fifteen years, five months (Napier and Napier, 1967).

In view of the present trend toward the use of primates in research, and the paucity of information regarding the reproductive physiology of the species under consideration, the present studies were proposed and designed according to the following objectives:

1. The characterization of basic reproductive parameters of *M. fascicularis* in a caged environment; viz. the length of the menstrual cycle; the duration and intensity of menstrual bleeding; reproductive behavior in controlled matings; temperature variation in the menstrual cycle; the length of gestation; and implantation bleeding.
2. The development and application of laparoscopic techniques to facilitate observation and photography

of the reproductive organs; and the recovery of follicular contents via follicular puncture.

3. The identification of external ovarian morphology near ovulation as a means of accurately predicting the time of ovulation.

REVIEW OF LITERATURE

The literature will be reviewed under three main headings: (1) the characterization of normal reproductive parameters in *M. fascicularis*; (2) the laparoscope: development, application, and technique; and (3) ovulation induction and follicular morphology in *M. fascicularis*. Literature regarding *M. Mulatta* will be cited where information regarding *M. fascicularis* is lacking, or where the comparison is deemed appropriate.

Characterization of Normal Reproductive Parameters in *Macaca Fascicularis*

The topic of reproductive parameters will be reviewed in four sections: (1) the length of the menstrual cycle and the duration and intensity of menstrual flow; (2) the systematic evaluation of reproductive performance in controlled matings; (3) basal temperature variation in the menstrual cycle; and (4) optimum breeding time, implantation bleeding, and gestation length.

Length of Menstrual Cycle, and Duration and Intensity of Menstrual Flow in *M. mulatta* and *M. fascicularis*

M. mulatta.--The first systematic description of menstruation in *M. mulatta* was provided by J. B. Sutton (1880). He reported simply that "Macacus" menstruates fairly regularly, and that there is a discharge of blood. Rhesus monkeys and baboons were sacrificed during the phase of uterine bleeding to determine if the bleeding was associated with endometrial destruction. He falsely concluded that there was no shedding of the uterine endometrium, but that the blood merely escaped from a congested mucous membrane.

The foundation of modern knowledge of the menstrual cycle of nonhuman primates was established by Walter Heape. Heape (1894) provided an account of his investigation of the uterine endometrium during various stages of the menstrual cycle in *Semnopithecus entellus* (a langur, genus Presbytis, probably the Hanuman or Entellus langur), and in *M. mulatta*. He did not keep accurate records of cycle length and menstrual flow, but referred to menstruation as occurring for about four days each month in *M. mulatta*.

In 1897, Heape reported that the mean cycle length for four cycles observed was 29.5 days, with the menstrual flow lasting 3 to 5 days. He also noted the external signs of menstruation in *M. mulatta*, viz. swelling and congestion of the nipples and vulva, and swollen red skin on the buttocks. Heape further discussed the nature of the menstrual

discharge in very descriptive terms. He stated that the discharge consisted of "a somewhat viscid, stringy, opaque fluid, red blood corpuscles, masses of uterine stromal tissue . . . and leucocytes."

In his paper on the "sexual season" of mammals (1900), Heape defined the terms breeding season, sexual season, proestrus, estrus, metestrus, and anestrus; and noted that most monkeys cycle throughout the year, that monkeys and human females are the most polyestrus mammals, and that zoo macaques have estrous periods of 2 to 3 days.

Allen (1927) subsequently reported that menstrual cycles in *M. mulatta* varied from 10 to 70 days; and that the normal menses had a modal length of 4 days.

The technique of bimanual rectal palpation was introduced by Hartman in 1933 to estimate the time of ovulation in *M. mulatta*. The correlation between time of ovulation and menstrual cycle data provided the first measurements of the lengths of the follicular and luteal phases in the primate reproductive cycle. The mean cycle length in the 50 cases reported was 25.3 days; the mode, 21 days; and the range, 20 to 32 days. He observed that ovulation occurred near mid-cycle, and that the ratio of the pre-ovulatory interval to cycle length was far more constant than the ratio of the post-ovulatory interval. The interval from the first day of menstruation to ovulation ranged from 9 to 17 days; whereas the interval from ovulation to the

first day of succeeding menstruation ranged from 6 to 23 days, with a mean of 14 days.

Bryans (1951) reported that two *M. mulatta* had average cycle lengths of 25.6 and 26.2 days.

During exposure of an experimental group of *M. mulatta* to an altered light-dark periodicity (Erickson, 1961), a total of 56 menstrual cycles were reported. The cycle lengths of control (normal day-night cycles) and experimental groups were remarkably uniform: 28.4 ± 2.54 days for the controls; and 29.4 ± 2.18, and 30.15 ± 5.95 days for the experimental groups, which led Erickson to conclude that the altered light-dark rhythm did not have a significant effect on the length of the menstrual cycle.

Van Wagenen (1966) cited 28 days as the modal length of the menstrual cycle for this species while Pickering, in the prepared discussion of van Wagenen's paper, stated that analyses of 450 cycles of 30 animals provided a mean cycle length of 29 ± 3 days, and vaginal flow of 4 ± 1 days.

Data on the menstrual periodicity of *M. mulatta* was again reported by Eckstein et al. (1969), wherein a total of 532 menstrual cycles were observed in 84 animals providing a mean cycle length of 28.8 days.

Monroe et al. (1970) have provided a quantitative description of the circulating levels of luteinizing hormone (LH) in *M. mulatta* during the menstrual cycle. A portion of their results described the relative variability of the follicular and luteal phases. The number of days following the

LH surge was found to occur 15 days after the LH surge in 7 of the 9 cases reported. The period of time from day one of the menstrual cycle to the time of the LH peak ranged from 9 to 16 days, with the highest frequency (5 animals) between days 10 and 12.

Investigating the annual changes in the menstrual pattern of *M. mulatta*, Keverne and Michael (1970) observed 223 menstrual cycles and reported the mean cycle length for all cycles as 38.6 ± 1.6 days, with a range of 17 to 158 days; and a modal length of 28 days, with 48.2% of all cycles between 27 and 31 days in length. The mean cycle length for the months December to April was 31.2 ± 0.8 days, whereas the mean for the period June to October was 56.6 ± 5.9 days. These observations indicated a high incidence of summer amenorrhea (referred to falsely, in some references, as "summer sterility") and long cycles in a laboratory colony in London.

Macdonald (1971) has recently reported the mean length of the menstrual cycle of *M. mulatta* in 74 cycles observed to be 29.2 ± 0.7 days. The mean length of menstrual flow was reported to be 3.3 ± 0.1 days.

M. fascicularis.--In his report on menstruation and ovulation in "Macacus rhesus," Heape (1897) provided the first recorded observations of cyclicity in *M. fascicularis*. He stated that he observed a "specimen of *Macacus cynomolgus*" which menstruated regularly for three months, each cycle being 31 days in length. He also cited congestion of the

nipples, vulva, and buttocks as characteristic of this species near the time of menstruation.

The cyclicity of *M. fascicularis* is also cited in the early literature by Van Herwerden (1906). She referred to this species as *Cercocebus cynomologo*, and was the first to speculate that a distinctive feature of macaque reproduction may be a high frequency of menstruation without ovulation. This observation has since been confirmed by other investigators (Hartman, 1939; Dempsey, 1939; Eckstein, 1949; Lang, 1967).

Joachimovits (1928) discussed the reproductive physiology of a species which he called *Pithecus fascicularis (mordax)*, which is considered to be a synonym of *M. fascicularis*. He stated that the animals he observed menstruated every 25 to 29 days, but showed wide variations of up to 35 days. He observed that menstrual flow usually lasted 3 to 5 days.

Spiegel's report (1930), based on the observation of 47 cycles in 4 *M. fascicularis*, found the modal length of the menstrual cycles to be 30 days.

In Corner's report (1932) on the menstrual cycle of the Malayan monkey *(M. fascicularis)*, he noted that the animals showed relatively regular cycles during the winter, and tended to amenorrhea in the summer. The modal length was reported as 35 days, the mean 40.1, and the range, 24 to 52 days. He concluded that the menstrual cycle length of *M. fascicularis* was longer than that of *M. mulatta* by at least

3 or 4 days. Menstrual flow in the 4 specimens that Corner observed lasted from 2 to 13 days, with a mode of 4 days.

Due to the overwhelming popularity of *M. mulatta* in primate research, from 1930 through 1960, there was very little published concerning *M. fascicularis*. In 1966, Fijiwara and Imamachi attempted to establish a breeding colony of *M. fascicularis* for medical research. They observed 175 menstrual cycles in the 40 females in their colony. The cycles ranged from 13 to 178 days with the majority of cycles in the 23 to 37 day range. A modal length was not indicated.

In a comparison of the macaque menstrual cycles, Kerber and Reese (1969) determined the lengths of 226 *M. fascicularis* and 523 *M. mulatta* menstrual cycles over a 4 year period. The length of the *M. fascicularis* menstrual cycles ranged from 9 to 339 days, with a mean of 47.2 days, a median of 32 days, and modes of 26, 27, and 28 days. Twenty-six percent of these cycles were 26 to 30 days in length. A total of 452 menstrual periods were observed in *M. fascicularis*. The mean duration of the menstrual bleeding was 2.6 days, while the median was 2 days. The length of the menstrual bleeding in *M. fascicularis* ranged between 1 and 16 days, with a single day of flow being the most common. In comparison to *M. mulatta*, they concluded that *M. fascicularis* has a cycle length slightly shorter and more variable than the 28 day cycle of *M. mulatta*. The duration of menstrual bleeding was found to be identical.

Mahoney (1970), in his efforts to detect ovulation in *M. fascicularis*, took daily vaginal smears beginning on the first day of the menstrual cycle. He found that menstrual bleeding usually lasted 1 to 6 days, with 1 to 3 days of occult flow preceding this in a small number of cases. This pointed out the possibility of a miscalculation of up to 3 days in assessing the first day of the menstrual cycle in those animals whose menstrual bleeding begins with an occult phase.

Macdonald (1971) indicated a mean of 31.3 ± 1.5 days for 21 menstrual cycles in *M. fascicularis* with a mean duration of menstrual flow of 4.2 ± 0.3 days for 35 observations.

Using laparoscopy to diagnose ovulation in conjunction with menstrual cycle records, and as a preliminary report of the present research, the mean cycle length of 31 cycles in *M. fascicularis* was determined by Jewett et al. (1971) to be 28.6 days. The mean length of the luteal phase was found to be 13.6 days, while the more variable follicular phase was found to be 15.0 days.

Systematic Evaluation of Reproductive Performance in the Macaque in Controlled Matings

The first published study concerning the reproductive behavior of the macaque was completed by Ball and Hartman in 1935. Their objective was to correlate vaginal smears and ovulation as determined by rectal palpation with the level of sexual excitation in the female *M. mulatta*.

A pre-ovulatory increase in sexual activity was observed, but a pre-menstrual increase did not appear.

Until the work of Michael and Herbert (1963), the role of ovarian hormones as a determining factor in sexually related behavior received little attention. Michael found that the peak in mounting and grooming activity of the male occurred near the expected time of ovulation, and that all mounting and grooming cycles were abolished by bilateral ovariectomy. He concluded that the endocrine status of the female may have considerable influence on heterosexual interactions in *M. mulatta*.

The first attempt to systematically evaluate the influence of endocrine factors upon various aspects of sexual and social behavior in *M. mulatta* was undertaken by Michael in 1965. He observed males and females in glass-fronted observation cages and a system was devised to score individual components of male and female behavior. Through bilateral ovariectomy and progesterone and estrogen replacement therapy, he determined that copulatory and grooming behavior, initiation and occurrence of mounts, thrusts, and ejaculations, as well as various other aspects of reproductive performance were markedly influenced, and to some degree determined, by the endocrine state of the female.

Michael et al. (1966a) used six pairs of adult *M. mulatta* to further demonstrate the effect of endocrine factors on primate sexual behavior. Bilateral ovariectomy again resulted in a notable reduction in all the measures

of sexual activity. The administration of estradiol, either subcutaneously or directly into the brain, restored sexual interest. Restoration of mounting activity and the ejaculatory capacity of the males were notably diminished by the administration of progesterone to the females.

Similar results were obtained by Michael et al. (1966b) when grooming behavior was studied qualitatively by observing pairs of adult *M. mulatta* during regular hourly test sessions. Rhythmic fluctuations in both male and female grooming occurred in relation to the menstrual cycle. The grooming time of males reached a maximum near mid-cycle, at which time the female's grooming was at a minimum. Bilateral ovariectomy of the female abolished all rhythmic variations and reduced the grooming of the males. Subcutaneous injections of estradiol into the ovariectomized female restored the grooming of males to the levels near mid-cycle. The subsequent addition of progesterone antagonized the effects of estradiol.

A report by Erikson (1967) utilized a vaginal cannulation technique to evaluate sexual receptivity of female *M. mulatta* throughout the menstrual cycle. A positive vaginal response was scored when a tightening of the vaginal tract occurred upon insertion of a glass cannula. An increase in vaginal responses and receptivity was reported not only during the probable time of ovulation but during the pre-menstrual period as well.

The relation between mounting and thrusting activity and the timing of ejaculation in male *M. mulatta* was subsequently investigated by Michael and Saayman (1967). Six pairs of adult monkeys were observed during mating tests, each of 60 minutes, before and after bilateral ovariectomy of the female of the pair. Components of behavior were quantified by means of a scoring system, with particular attention given to the following parameters: (a) ejaculation time, i.e., the time from the first mount of a mounting series to the occurrence of ejaculation; (b) mounts to ejaculation, i.e., the number of mounts in a mounting series preceding the first ejaculation; (c) total thrusts to ejaculation, i.e., the number of pelvic thrusts from intromission to the first ejaculation. From these measurements, the following values were derived: (1) the mounting rate, i.e., the number of thrusts to ejaculation divided by the ejaculation time; and (2) the thrusts per mount, i.e., the total number of thrusts to ejaculation divided by the number of mounts to ejaculation. There was found to be a clear relation between ejaculation time and the product of mounting rate times thrusts per mount. The latter measurement was then adopted as the sexual performance index (SPI). The SPI was observed to decline markedly as ejaculation time increased, and different males were found to have widely differing ejaculation times.

Michael et al. (1967a) further established the influence of the secretory activity of the female's ovaries on

the sexual behavior of the male by observing the rhythmic fluctuation of the mating behavior of the males in relation to the menstrual cycles of their female partners. Two patterns were observed: one with a high level of mounting in the follicular phase and a low level in the luteal phase, and another with maximum mounting near mid-cycle. Ejaculation times were shortest and mounting rates highest near ovulation.

Ovariectomized *M. mulatta* were again treated with estradiol and progesterone (Michael et al., 1967b), with particular attention given to (a) the number of sexual mounts made by the male upon the female per test, and (b) the number of active refusals per test made by the female when the male attempted to mount. They concluded that progesterone inhibited sexual receptivity in the female by activating a refusal mechanism; and that this could account, in part, for the decrease in mounting activity of the males, tested with intact females, observed during the early part of the luteal phase of the menstrual cycle.

Further clarification of male-female sexual interaction was achieved by Michael et al. (1968), again using ovariectomized, estrogen treated females which were given subcutaneous injections of progesterone. They concluded that the loss of female receptivity and loss of female attractiveness, previously observed during the luteal phase of a female's menstrual cycle, were reproduced by giving progesterone to the ovariectomized, estrogen treated partners.

Grooming patterns, similar to those described in *M. mulatta* by Michael et al. (1966) were observed in adult baboons *(Papio sp.)* by Rowell (1968). He found that females were groomed more frequently by males in the late follicular phase of their menstrual cycles, thereby adding to the evidence which suggested that a high degree of correlation exists between the hormonal status of the female and the sexual interactions of the female with her partner.

The suggestion that a pheromone-like substance may be responsible for the variations in sexual activity of *M. mulatta* was supported by Michael and Saayman (1968) with the subcutaneous and intravaginal administration of estrogen. The intravaginal route resulted in a greater stimulation of the male's interest and in the number of male mounting attempts. It was concluded that the communication between these primates was influenced by estrogen-dependent changes in the vagina.

The work of Everitt and Herbert (1969), using estrogen-treated ovariectomized females *(M. mulatta)* demonstrated that it was possible to change the male monkey's sexual preference by altering the hormonal condition of the female.

Vendenbergh (1969) then provided evidence that sexually quiescent male monkeys *(M. mulatta)* can be returned to a sexually active state by exposure to females artificially brought into estrus. Males responded behaviorally by showing copulatory activities, and by increased

duration of grooming; and physiologically by showing evidence of increased testicular activity as determined by histological examination of testicular biopsies. He concluded that such behavioral and endocrine coordination pointed out (1) the control hormones exert over primate sexual behavior, (2) that females communicate their endocrine states to males, and (3) the existence of a system for the synchronization of mating activities between the sexes, especially at the onset of the breeding season.

In a subsequent paper by Missakian et al. (1969), patterns of reproductive behavior among male *M. mulatta* in captivity were investigated on a long-term basis. The results of this study indicated that over a successive series of mounts, the following changes occurred: (1) increased mount frequency, (2) increased ejaculation time, (3) increased intermount interval, (4) decreased thrusts per mount, and (5) no change in total thrust frequency. These findings suggested that an increase in mounting frequency was associated with a dissipation of sexual excitation during the intermount interval.

Basal Temperature Variation in the Macaque Menstrual Cycle

Although the first successful attempt to correlate changes in basal temperature with events in the menstrual cycle was made by Squire (1868), the study conducted by Simpson and Galbraith (1906) is generally considered to be the first systematic, rigorous investigation of temperature

fluctuations in the primate menstrual cycle. The macaques used in this study were *M. mulatta* and *M. fascicularis*, and temperatures were taken from the rectum and axilla. They established a mean diurnal variation of 2 to 3 degrees Centigrade in both species; and a mean rectal temperature of 38°C in *M. mulatta*, whereas *M. fascicularis* ranged from 0.2°C to 2.0°C lower. It was also concluded that basal temperature is more subject to variation by environmental influences in *M. mulatta* and *M. fascicularis* than in man.

Erickson (1960) reported diurnal temperature variations in the adult *M. mulatta*, where vaginal temperature recordings were made routinely with a clinical thermometer. Temperature variations were not correlated with menstrual cycles, but the establishment of diurnal fluctuations was a necessary prerequisite to the evaluation of temperature recordings in the study of cyclicity. The diurnal range was found to be 3°F with the highest temperatures recorded early in the afternoon, and the lowest recorded at 10 P.M.

Attempting an accurate estimate of the moment of ovulation by monitoring temperature, Balin and Wan (1968) used a telemetry system wherein a subminiature telemeter was implanted in the abdomen of *M. mulatta*. The temperature sensing device in the implant was positioned near the ovary so that not only could general changes in body temperature be monitored, but localized differences in the temperature of the ovarian surface could also be detected which might define ovulation. From the body temperature curves obtained,

it appeared that a biphasic temperature pattern did indeed correlate with the ovulatory cycle as determined by laparotomy, ovarian biopsy, and vaginal cytology. They also found a diurnal fluctuation of 2° to 3°F as did Erickson (1960), and recognized therefore, that subtle temperature changes which might be related to ovulation would be difficult to detect. It was found, however, that there was apparently a smaller day to night temperature difference in the pre-ovulatory phase than in the post-ovulatory phase. Furthermore, the rate of change of temperature with time at the hours of "light-on" and "light-off" was observed to be more rapid in the follicular phase than in the luteal phase. Although elaborate analysis of the telemetry data did indicate definite trends in deep-body and ovarian surface temperature, this type of information could not be used to reliably predict or identify the moment of ovulation.

Optimum Breeding Time, Implantation Bleeding, and Gestation Length

Optimum breeding time.--Lewis and Hartman (1933) reported an observation of ovulation by rectal palpation of a female *M. mulatta* on day 15 of the menstrual cycle. During this menstrual cycle a male was continually present with the palpated female, and she did conceive during the cycle, presumably on day 15. The average length of the menstrual cycle for this animal was not indicated.

Van Wagenen (1945a) reported a controlled breeding experiment with *M. mulatta* which resulted in 160 pregnancies. The 24 hour period common to the greatest number of these matings which were followed by pregnancy extended from noon of the eleventh day of the menstrual cycle to noon of the twelfth day. Fifty-six percent of the animals became pregnant after two matings; 32% with a single mating.

The optimum breeding time for *M. mulatta* as established by Jacobson and Windle (1960) was on the ninth day for females with cycles of 25 days or less; on the tenth or eleventh day in the case of 26 to 28 day cycles; and on the twelfth day for cycles of 29 days or more.

Wilson et al. (1970) used the "calendar" method of van Wagenen (1945b) in which the optimal breeding time was based on the statistical probability that the modal time of ovulation in *M. mulatta*, maintained in the laboratory, is day 12 of the menstrual cycle. In this study, females were placed in a cage with a male for a period of 48 hours extending from 10 A.M. of day 11 to 10 A.M. of day 13. Of 17 females under observation for 26 months, all became pregnant by 24 months after the first observed menstruation.

Valerio (1970) arranged an optimum mating schedule (Table 1) for *M. mulatta* based on differences in cycle length, similar to the method of Jacobson and Windle (1960).

Table 1. Mating schedule for females based on menstrual cycle length (Valerio, 1970)

Menstrual cycle(s) (days)	Days of cycle[a] mated
17-19	7-10
19-21	8-11
22-23	9-12
24-25	10-13
26-30	11-14
31-33	12-15
34-36	13-16
37-40	14-17

[a]Day 1 = first day of menstruation.

Although the conception rate for each category was not reported, it was found that the animals who conceived most commonly had cycle lengths of 28 and 30 days, whereas few pregnancies occurred after menstrual cycles of 15 through 22 days, and from 37 through 50 days. Females with cycle lengths of 23 to 35 days had the highest incidence of pregnancy. It was noted that the modal length of menses in which conception occurred was 4 days, and that very few conceptions resulted during cycles in which the duration of menses was greater than 7 days. The mode for the number of matings per pregnancy was 3, while 69% of females required 5 or less matings, and less than 3% required between 14 and 21 matings.

Nine births within 43 days resulting from 36 matings indicated a high conception rate in *M. fasciularis* (Dukelow,

1970) when matings were accomplished by daily exposure to males for 2 hours from day 11 to day 15 of the menstrual cycle.

Implantation bleeding and gestation length.--Spiegel (1954) has cited gestation lengths in *M. fascicularis* ranging from 153 days to 179 days, with a mean of 167 days.

In 1966, Fujiwara and Imamichi calculated the length of gestation in *M. fascicularis* as between 153 and 225 days with a mean of 168 days. It was noted that the range of 72 days was approximately twice the length of the menstrual cycle.

Macdonald (1971) found the gestation length of *M. mulatta* and *M. fascicularis* to be essentially the same: 165.7 ± 1.5 days (12 observations, *M. mulatta*), and 162.7 ± 0.9 days (10 observations, *M. fascicularis*). He also observed that the incidence of implantation bleeding was greater in *M. fascicularis* than in *M. mulatta*. This phenomenon was observed in 14 of 15 pregnancies in *M. fascicularis*, and lasted for 19.1 ± 2.0 days; whereas in *M. mulatta*, implantation bleeding was observed in 5 of 10 pregnancies and the duration of flow was given as 10.8 ± 3.6 days. The onset of implantation bleeding occurred during the third week of pregnancy in both species.

The Laparoscope: Development, Application, and Technique

The earliest known report of endoscopy is that of Bozzini (1806) who described the projection of candlelight through a double-lumen urethral cannula. The term endoscopy did not appear in the literature, however, until 1867, when it was used by Segelas and Desormeaux in their description of light passage through a genito-urinary speculum (cited in Benedict, 1951).

The invention of the incandescent light bulb and its incorporation into the distal tip of the endoscope eventually led to the type of apparatus that is still in use by the majority of endoscopists. The first successful illumination of an internal body cavity (the bladder) using an incandescent light source is credited to Max von Nitze (1894), and it was he who took the first endoscopic photographs through the prismatic cystoscope.

Endoscopic visualization of the general peritoneal cavity can be traced to the contributions of Kelling (1902), and Jacobaeus (1910) that culminated in the development of a safe technique for inserting an optical telescope through a small abdominal incision. It was Jacobaeus who coined the term laparoscopy. The endoscope was subsequently introduced to the United States by Bernheim in 1911.

Nordentoeft (1912) was the first to describe endoscopic views of pelvic organs obtained by this technique.

The next significant development was the universal endoscope of Fourestier (1960) which eliminated several of the disadvantages of distal bulb illumination through the utilization of the principle of proximal light projection. Illumination by this technique is conveyed from a proximal light source by a clear rod of fused quartz which not only removed the threat of heat-burn, but also furnished a more brilliant illumination than previously available. The Fourestier universal endoscope has not been generally accepted in this country because of its cost, the fragility of the quartz rod, and because of the proximity of the externally housed light bulb to the eyepiece which makes endoscopic manipulation somewhat cumbersome.

During this time there were several attempts to construct fiber optic endoscopes. The construction of such devices was based on the phenomenon of the conduction of light along a transparent fiber cylinder as a result of internal reflectance.

Extensive clinical testing of the application of fiber optic systems to the laparoscope was reported by Fear (1968) wherein he described the results of 134 examinations performed under general anesthesia. He emphasized the safety and convenience of laparoscopy in gynecologic procedures. Fear also provided a chronology of the development of the modern laparoscope which is of interest in this review.

Historical Perspective (Fear, 1968)

1901 Peritoneal cavity of dog visualized with modified cystoscope (Kelling, Germany).

1910 First clinically useful laparoscope (Jacobaeus, Germany).

1911 First use of laparoscope in America (Bernheim).

1912 Pneumoperitoneum and Trendelenburg position introduced (Nordentoeft, Denmark).

1913 Widespread use of laparoscopy in Europe and Scandinavia.

1928 First high-quality instrument devised (Kalk, Germany).

1937 First large series reported from U.S.A. (Ruddock); use in ectopic pregnancy stressed (Hope, U.S.A.); tubal sterilization performed through laparoscope (Anderson, U.S.A.).

1942 Technique for uterine suspension through laparoscope described (Donaldson, U.S.A.).

1944 Extensive use of biopsy forceps through laparoscope (Palmer, France).

1952 Remote light source of high intensity developed (Fourestier, Gladu, and Vulmiere, France).

1955 Lens systems improved and modern instruments available.

1956 Color photographs taken through laparoscope.

1959 Closed circuit television through laparoscope.

1960 Tantalum clips applied across tubes for sterilization (Neumann and Frick, U.S.A.).

1961 Textbooks on laparoscopy written in German, French, and Italian.

1964 First international symposium on laparoscopy (Italy).

1967 First textbook written in English (Steptoe, London).

Although many investigators pioneered the development of the fiber optic laparoscope, many of the refinements which led to its current popularity are attributed to Semm (1969, 1970). He outlined the surgical procedure in detail, cautioned about potential hazards, and developed the instrumentarium for a variety of procedures such as uterine biopsy and tubal sterilization.

A comprehensive list of indications, contraindications, complications, and case studies involving the use of the laparoscope has been provided by Smith and Dillon (1970). It was noted in their report that an obvious advantage of laparoscopy is that it can be performed in cases where contraindication due to disease or abnormal anatomy preclude culdoscopy.

A repertory of ancillary techniques which can be performed by laparoscopy has been described by Siegler and Garret (1970). These techniques include aspiration, insufflation, lysis of adhesions, and coagulation.

Steptoe and Edwards (1970) have described the detection of ovulation in humans, as well as the aspiration of pre-ovulatory oocytes with the laparoscope.

The use of the laparoscope to determine gestation length, diagnose pregnancy, and detect impending ovulation in the macaque has been described by Jewett and Dukelow (1971).

Ovulation Induction and Follicular Morphology in the Macaque

Ovulation Induction

For a number of years, hypophyseal extracts had been used to stimulate follicular growth in *M. mulatta* (Hartman and Squire, 1931; Hisaw et al., 1931; Hisaw et al., 1935). The establishment of a reliable ovulation induction regime, however, began with the efforts of van Wagenen and Simpson in 1957. In their experiments, they used monkey pituitary extract supplemented with human chorionic gonadotropin (HCG) which resulted in 1 to 12 ovulations per ovary in adult *M. mulatta*. From the data in this report came the first suggestion of species specificity regarding the effects of gonadotropins in the attempt to induce ovulation in the macaque.

In further trials, Simpson and van Wagenen (1958) determined that the time between the onset of therapy with monkey pituitary extract (3 to 5 mg per day for 8 to 10 days) and ovulation (determined by the structure of the corpora lutea) was 5 to 7 days.

Multiple ovulations were induced by van Wagenen (1962) in *M. mulatta* following injection of human postmenopausal gonadotrophin (HMG). Luteinization was achieved using HCG. It was speculated that incorrect dosage and timing may have contributed to the multiple ovulations (3 to 4 ova per ovary).

Mastroianni and Rosseau (1965) used the method of Simpson and van Wagenen (1962) to superovulate *M. mulatta* in order to evaluate the effect of the Margulies type of intra-uterine device (IUD) on ovum transport. Their induction regime consisted of 550 follicle stimulating hormone (FSH) units of human urinary gonadotropin (HMG, Pergonal) given intramuscularly (IM). This was administered daily for 6 days beginning on day 5 of the menstrual cycle. This was followed by 275 units of HMG and 2,000 International Units (I.U.) of HCG for 3 days. This resulted in a total of 36 ovulation points in 15 animals.

In a program to improve the pregnancy rate in a colony of *M. mulatta*, Dede and Plentl (1966) artificially inseminated monkeys which had been induced to ovulate. They induced ovulation using the method of van Wagenen (1962), but began the injections of HMG on days 2 or 3 of the menstrual cycle. Ovarian enlargement as determined by rectal palpation, hyperemia, and edema of the perineal "sex skin" were accepted as evidence of a response to HMG treatment. At this point, 2,000 I.U. of HCG were added to the HMG for 2 days. Although ovulation was induced in 31 cycles, the success of this regime on a "per trial" basis, and the incidence of multiple ovulations were not indicated.

Balin and Wan (1968), in their investigation of possible local thermal changes on the ovarian surface induced by ovulation, utilized the HMG-HCG regime of van Wagenen (1962) to induce ovulation in otherwise anovulatory

adult *M. mulatta*. Ovulation was confirmed by the recording of a temperature dip on the last day of gonadotropin therapy, followed by a sustained temperature rise, by vaginal cytology, and by laparotomy. Again, the success rate was not indicated.

Wan and Balin (1969) undertook a series of experiments using HMG-HCG, clomiphene citrate, and dl-18-methylestriol to evaluate their relative effectiveness as ovulation inducing agents, and to find a regime which would avoid the undesirable ovarian hypertrophy of existing regimes.

HMG-HCG.--Two dosage regimes were used in this study. Schedule 1: 75 I.U. of Pergonal given IM for 5 to 8 days, followed by 35.5 I.U. of Pergonal and 2,000 I.U. HCG for 3 days. Schedule 2: one ampule (75 I.U.) Pergonal for 6 to 7 days followed by a single large dose of HCG (5,000 I.U.) given intravenously (I.V.). A total of 23 female *M. mulatta* were treated with these regimes for a total of 30 cycles. The results are indicated below in Table 2.

Table 2. Ovulation induction in *M. mulatta*[a]

Schedule	Number of cycles treated	Ovulations induced
1	23	13 (56.6%)
2	7	5 (71%)
Total	30	18

[a]Source: Wan and Balin, 1969.

The HMG/HCG regime was successful in 60% of the cycles; while Schedule 2, with a single large I.V. dose of HCG, proved to be slightly more effective. The authors also indicated that Schedule 2 permitted the prediction of ovulation time with greater accuracy.

Clomiphene citrate.--A total of 15 *M. mulatta* were used in this study. Four were treated with a racemic mixture (cis and trans clomiphene citrate) and 5 with each isomer. Clomiphene was given for 5 days at doses ranging from 0.2 mg/Kg/day to 1.5 mg/Kg/day. No differences were found in the effectiveness of either isomer, or in the racemic mixture. Ovulation was successfully induced in at least one treatment cycle in 14 of the 15 monkeys (94%); although, of the 46 cycles treated, 59% ovulated, which was comparable to the HMG/HCG regime.

dl-18-methyl estriol.--A total of 9 *M. mulatta* were used in this study, and a dose of 5 mg per day was given orally for 5 days in the first treatment cycle. The dosage was then increased to 10 mg per day in the second cycle if ovulation had not been induced at the 5 mg level, and finally to 20 mg per day if the 10 mg level failed to induce ovulation. Treatment was begun on day 5 of the menstrual cycle. It was found that while ovulation was induced in at least one treatment cycle in 6 of the 9 monkeys (67%), only 32% of the total of 26 treatment cycles resulted in ovulations. Ovulation was not enhanced by increased doses.

It was noted that HMG/HCG treatment resulted in 46% multiple ovulations, whereas all of the ovulations with clomiphene citrate were single ovulations, and no ovarian cysts were found.

A report by Mahoney (1970) indicated that in *M. fascicularis* which had failed to respond to a regime of clomiphene citrate administered in doses of 3 to 5 mg/Kg/day for 5 days beginning on day 5 of the menstrual cycle, the administration of pregnant mare serum gonadotropin (PMSG) and HCG would result in ovulation.

Follicular Morphology

Blandau has furnished an excellent description of the anatomy of ovulation in the rat (Blandau, 1955), in which he cites the appearance of stigmata as the first indication of impending ovulation. He noted a thin secondary cone which ruptured during ovulation, and described the cone as a circumscribed avascular area. Ovulation was marked by the escape of follicular fluid and gradual collapse of the follicular walls. Blandau estimated that the time required for ovum extrusion, once the stigma had ruptured, ranged from 11 seconds to 12 minutes.

Betteridge et al. (1970), in the examination of 121 menstrual cycles in *M. mulatta* by laparotomy, found that diagnosis of ovulation was difficult due to the variable appearance of ovulation points. It was noted, however,

that recent ovulation points were easily diagnosed if the follicle was open, bleeding, and had a distinct irregularity in the follicular wall.

METHODS AND MATERIALS

History of the Macaque Colony

The original colony of ten female and six male Cynomolgus macaques at Michigan State University was purchased from the Detroit Zoo in January, 1968. Thirty-six monkeys were added in March, 1968; and, with three deliveries, the total in February, 1969 was twenty-four males and thirty females. The monkeys were housed at the Veterinary Research Farm on the Michigan State campus until February, 1969, when they were transferred to the Endocrine Research Unit. A format for the management and utilization of non-human primates in the new facility was established in February, 1969, and was designed to provide a controlled environment and procedural guidelines for the use of the colony in research. The original intention had been to provide mature females and breeding males to the Department of Anatomy for teratologic studies. With the transfer to the Endocrine Research Unit, the research objectives were broadened to include studies in the areas of ovulation, fertilization, implantation, and gestation. In February, 1970, the date of initiation of the present studies, 30 mature females, 6 mature males, and 7 infants, were housed

in the macaque colony. The emphasis in research in the past year has been on studies related to ovulation.

Although part of the original purpose of the colony was to maintain a breeding colony to replace those animals required by various departments, such a design was not economically feasible, since the cost of maintaining a monkey until sexual maturity outweighs the price at which it can be purchased from suppliers.

Colony Management

Environmental Control

Temperature and relative humidity.--The room temperature was maintained between 21°C and 26°C. The relative humidity was maintained at 40% to 60%.

Air flow.--The flow of air in the colony provided 12 complete air changes per hour with no recirculation of room air. The circulating air in the colony was also filtered.

Caging.--All the macaques were housed in cages designed by the Center for Laboratory Animal Resources (C.L.A.R.) at Michigan State University. The cages were 48 inch double unit modular cages equipped with a squeeze-back apparatus. The false floor was constructed of flattened tubing which allowed urine and feces to fall through the cage bottom onto a surface which articulated with a flush-exhaust system, thereby permitting rapid cleaning of the entire unit. The construction of the cage permitted air

to flow through the cage providing adequate ventilation. Drinking water was supplied through an "ad libitum" bottle-tube system. Feed boxes were attached to the units in such a way as to facilitate feeding while decreasing the amount of feed that was thrown from the cage.

Daily Care and Maintenance

Each cage was hosed daily and each cage unit was cleaned in a sterilizing wash once each month. The animals were fed daily, in the afternoon, and received a diet of a commercially prepared monkey food (Wayne). Water bottles were cleaned and filled daily. The colony was checked weekly for signs of disease by a veterinarian. The animals were wormed at 6 month intervals with thiabendazole (Equizole, Merck) in the drinking water. Tuberculin tests were also conducted on a yearly basis. All professional and technical personnel who came into contact with the colony were cautioned about potential health hazards. In addition all personnel were subject to annual chest X-ray.

Cyclicity of *M. fascicularis*

Menses was detected by daily visual inspection of the cages. The presence of blood was noted, and the possibility of bleeding from trauma or disease was eliminated by using the squeeze-back to check the buttocks of the animal in question. The intensity of menstrual flow was noted and recorded for each animal. Spotting, usually on the perch, was classified as light flow; several areas of bloody

"patches" on the perch and the cage floor was considered moderate; and blood found over most of the floor and perch, and occasionally on the walls, was classified as heavy.

Onset of menses was considered the first day of the menstrual cycle. A minimum of 6 menstrual cycles were recorded for all of the females before the present studies were begun. The lengths of these cycles were averaged to provide a basis for projecting a date for the expected onset of the next menses. Regular and irregular cycles were classified on the basis of menstrual flow beginning within 2 days of the projected date.

Limited Duration Matings

In order to determine gestation length and optimum mating time, and to undertake a long-term study of the fertilizable life of the ovum in *M. fascicularis*, 30 minute mating periods were conducted on days 11 through 18 of the menstrual cycle. The day of the cycle on which an animal was mated was dependent on the average cycle length of the female. Those with 28 day cycles were mated on days 11 through 14; those with 30 day cycles on days 14 through 16; and those with 32 day cycles, on days 15 through 18. A separate cage behind a one-way mirror was maintained for all matings. After the male had been removed from the breeding cage, an artificial insemination rod was used to take a vaginal smear to check for the presence of sperm. If sperm were not found, the trial was rejected.

Gestation Length and Implantation Bleeding

Gestation length was considered to be the length of time from the mid-point of the mating session to the time of the delivery. If the delivery occurred between two observations, then the end-point for the consideration of gestation length was the mid-point between the last pre-delivery observation and the first post-delivery observation.

The duration of implantation bleeding was recorded, and the days of pregnancy on which it occurred were noted.

Ovulation Induction

In the attempt to induce multiple ovulations in *M. fascicularis*, the following regime was used on 3 macaques with histories of amenorrhea.

75 I.U. PMS/day x 6 days, beginning on day of cycle (I.M.) (Ayerst, Equinex).

38 I.U. PMS and 2,000 I.U. HCG, both given I.M. x 3 days.

All of the monkeys in this trial had been laparoscoped prior to the induction trial, so that the appearance of the ovaries after the PMS/HCG regime could be compared with a pre-induction standard. Post-induction laparoscopic examinations were begun on the day following the last injections of PMS and HCG.

Temperature and Cyclicity in *M. fascicularis*

Four *M. fascicularis* were removed from their cages daily for one menstrual cycle and manually restrained while the rectal temperature was measured. Each series of temperature recordings was begun on the first day of menses, and continued through the cycle to screen for possible temperature variations which might be associated with ovulation.

Ancillary Apparatus for Use with the Laparoscope

Since there were no previous reports describing the use of laparoscopy in studies similar to those considered in this report, it was necessary to design various types of ancillary equipment for long term observation of the ovaries.

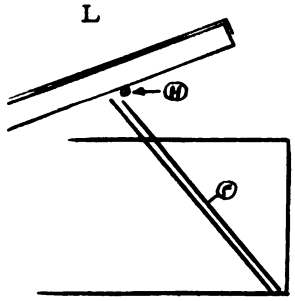
Laparoscopy Stage

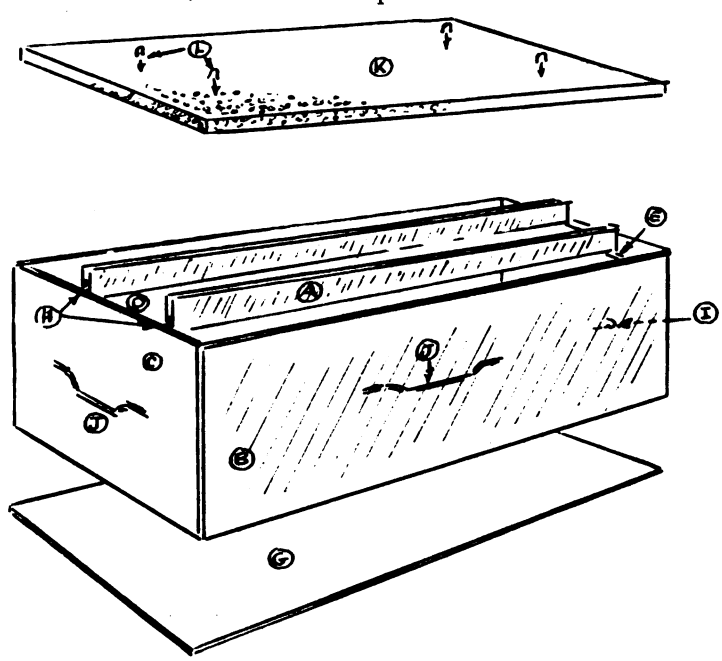
The materials for the construction of the variable-angle laparoscopy stage, and the procedure for assembly are illustrated in Figure 2. The dimensions of the stage, and the adjustability of the platform enabled the investigator to sit with the stage placed in front of him on a table of average height. The grid could be removed to facilitate cleaning. This design will support an animal up to 40 pounds.

FIGURE 2

VARIABLE ANGLE LAPAROSCOPY STAGE

Piece:	Quantity:	Description:
A	2	26 1/2" X 1 5/8" X 3 1/2"
B	2	26 1/8" X 9 3/8" X 5/8"
C	2	13" X 9 3/8" X 5/8"
D	1	26 1/2" X 9 1/2" X 5/8"
E	1	10" X 5/8" X 1"
F	1	15" X 5" X 5/8"
G	1	1/4" X 14 1/4" X 28 1/2"
H	3	2" Pin Hinges
I	1	Hook and Eye Clamp
J	4	6" Handles
K	1	Surgical Table
L	4	1/2" Metal Staples





Adjustable Clamp for the Laparoscopy Telescope

The adjustable clamp was improvised to provide a means of stabilizing the laparoscope for such procedures as comparative photography and follicular puncture. It has also been useful for laboratory demonstrations where a particular objective could be held in view for an extended period. Its construction and use are illustrated in Figure 3.

Suction Device and Recovery Unit for Follicular Puncture and Aspiration

A diagram of the follicular puncture and recovery unit, and the materials for its construction are provided in Figure 4.

Assembly of puncture and recovery unit.--A right angle bracket was secured to the right side of the surgical table mounted on the laparoscopy stage. A flow meter, sensitive in the range of 0.5 to 2.0 liters/minute, was then fastened to the bracket with the face of the meter toward the investigator. A curved pediatric ear-suction with a thumb cut-off (Frazier suction tube, #6 French, Lawton Co.) was equipped with a 14 gauge needle, the tip of which was slanted at 45 degrees and sharpened to a fine point. The needle was coupled to the ear-suction by using the tip of a disposable syringe. A 30 cm section of PE-90 polyethylene tubing (Clay-Adams, New York) was then flared at one end with an open flame, and placed within the needle and

FIGURE 3

ADJUSTABLE CLAMP FOR THE LAPAROSCOPY TELESCOPE

Piece:	Quantity:	Description:
A	2	Ring Stands
B	2	Clamp Holders
C	1	20" Rod
D	1	Clamp Holder With Hole
E	1	2" Piece P. E. Tubing (1/4" interior diameter)

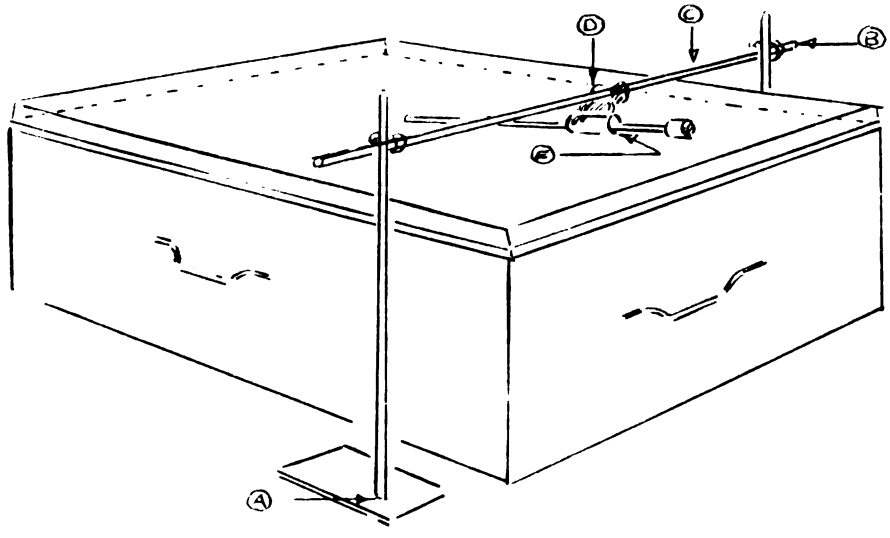


FIGURE 4

SUCTION DEVICE AND RECOVERY UNIT FOR FOLLICULAR PUNCTURE AND ASPIRATION

(1) Pediatric ear suction

(1) 14 gauge needle (modified)

(1) #90 P. E. tubing (30 cm)

(1) P. E. tubing, 1/4" I. D.

(1) #7 rubber stopper

glass tubing

(1) 500 ml. Erlenmeyer flask

(1) negative pressure pump

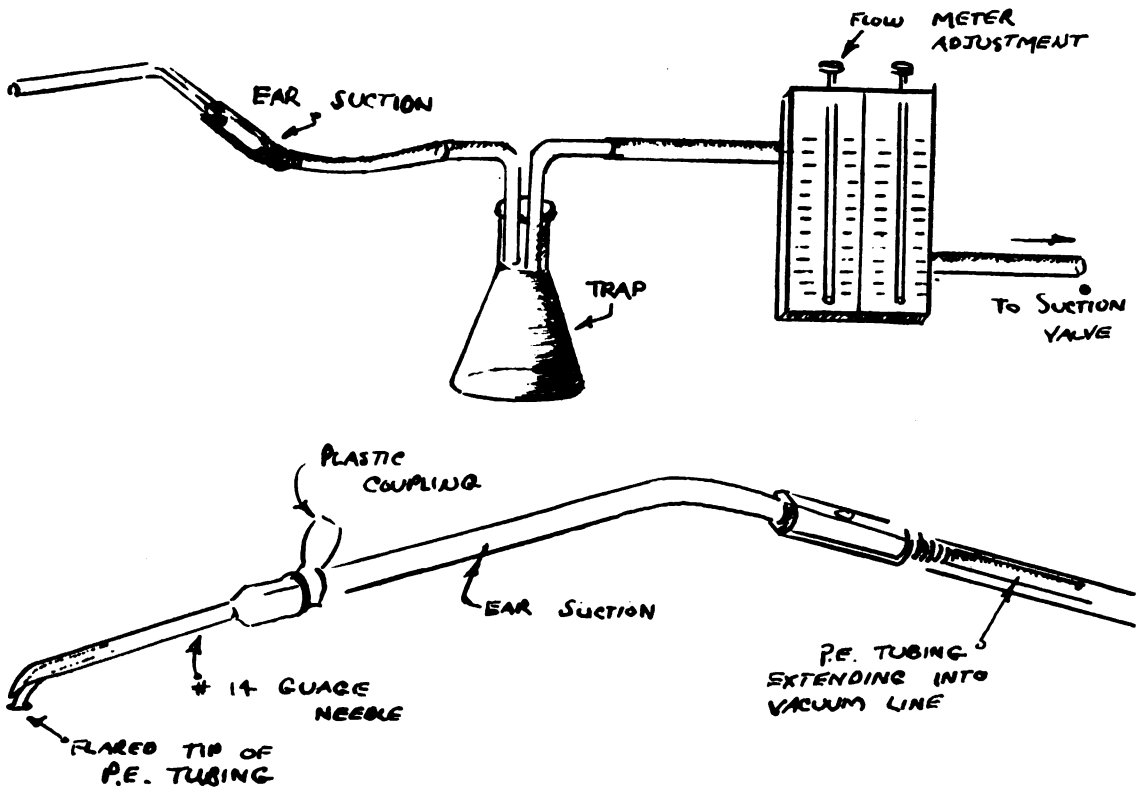
(1) Y connector

(1) microforceps

(5) 85 mm watchglasses

(1) 2. 5 ml. syringe

(1) 20 Gauge needle



ear-suction device, with the flared end exposed. The flared end permitted a good approximation of the tubing to the follicle and prevented it from being sucked into the ear-suction by the negative pressure. The assembled ear-suction device was connected to a trap made from a #7 rubber stopper, glass tubing, and a 500 ml Erlenmeyer flask. Finally, the trap was connected to the flow-meter, which was in turn connected to the vacuum pump.

Preparation of the Animal

When the monkey had been secured on the surgical table, a 1 cm incision was made on both right and left abdominal walls at a level 2 cm below the umbilicus. A #15 scalpel blade, when inserted through the incisions, facilitated the insertion of the cannulae. Each cannula was made from an arm of a Y-connector for polyethylene tubing. The ridges on the cannulae usually served well to hold it in place when inserted into the flank incisions. A purse-string suture was used if a cannula began to slip in the incision.

Evacuation of Follicles

When the follicle had been identified through the laparoscope, the telescope was secured in position with the adjustable clamp. The assembled suction unit was then inserted through one of the abdominal cannulae, while the microforceps were brought into position from the cannula on the opposite side. The ovarian ligament was then grasped

with the microforceps to stabilize the ovary for puncture. While maintaining the ovary in the most advantageous position, the tip of the needle was brought to the upper edge of the follicle. In this position, the flared end of the #90 polyethylene tubing articulated with the follicular wall. The air cut-off was then engaged and the follicle simultaneously incised. There was some inevitable loss of follicular fluid due to the sudden release of intrafollicular pressure, but most of the fluid was incorporated into the recovery tubing. A flow rate of 0.5 to 1.0 liters/minute provided sufficient negative pressure to evacuate the follicle without causing excessive trauma. The suction was discontinued when the follicular wall was collapsed and it appeared that extraneous material was being collected. The microforceps and suction unit were removed from the cannulae and the #90 tubing was removed from the ear-suction. A 2.5 ml syringe with a 20 gauge needle was inserted into one end of the tubing and the contents flushed into five 85 mm watchglasses. The use of several watchglasses facilitated examination of the aspirate because the follicular fluid could be separated from extraneous material which may have been collected at the end of the tubing, and the samples could be examined in the order in which they were collected.

Laparoscopy

The laparoscope consisted of a Wolf model 4000 projector light source, a fiber optic cable, and a 135 degree pediatric laparoscope 5 mm in diameter. The laparoscope, probes, and trocar-cannula were kept in benzalkonium chloride (Zephiran, Winthrop) prior to use (fiber optic systems cannot be autoclaved). The Squirrel monkeys *(S. sciureus)* were anesthetized with 15 mg sodium pentobarbital (Halatal, Jensen-Salisbery Laboratories) (I.P.) and the *M. fascicularis* were given phencyclidine HCl (Sernylan, Bio-Ceutic Laboratories) (I.M.) 0.15 mg/Kg. The animals were placed on their backs on the laparoscopy table, with the stage elevated at 45 degrees. The trocar-cannula was inserted through a 1 cm midline incision in the ventral abdomen. The trocar was then withdrawn, and the laparoscope inserted through the cannula. The fiber optic cable and the gas supply hose for abdominal distention were attached to the laparoscope and cannula, respectively. Periodic insufflation using 5% CO_2 in air, passed through a water chamber, was used to distend the abdominal cavity for better observation of the abdominal organs.

With abdominal distention, the reproductive organs could be visualized, and with the aid of a tactile probe inserted laterally, the organs could be manipulated.

Upon completion of the examination, a single suture was used to close the incision in *S. sciureus*, and subcuticular sutures were used in *M. fascicularis* to minimize the

amount of scar tissue and to expedite healing. Prophylactic injections of penicillin were given after the first examination of each series.

Laparoscopic Photography

In order to obtain satisfactory color photographs through the laparoscope, several combinations of films, cameras, and camera settings were tested. The following cameras were used: Exacta VX-1000, Olympus Pen-F, and a Canon TL. The cameras were attached to the telescope for laparoscopy by means of a 95 mm lens adapter. The following types of film were evaluated: Ektachrome EHB, Ektachrome X, Kodachrome X, Kodachrome KPA, and Anscochrome 500. Developing the exposed film at a higher ASA number (400 vs. 160) was also tried in the effort to produce better quality photographs.

For successful infrared photography, it was essential that the combination of light and filters be controlled very carefully. Ektachrome infrared film was used in all trials, in combination with the following types of filters: CC 20-C, Corning CS-1-59 (3966), Kodak Wratten #12, Wratten #87C, CC 30-C, Wratten #82A, CC20-B, #15G, CC10-C, and CC10 M. The setting #2 on the laparoscope light source was found to produce the proper light (3200°K) for use with Kodak infrared film.

Shutter speeds ranging from 1 second to 1/100 second were used with all combinations of film and light intensities.

Follicular Morphology

During the ovulation induction studies in *M. fascicularis*, follicles were observed at various stages of development, and it was recognized that distinctive markings appeared at various pre- and post-ovulatory stages. It was determined that identification of the morphology of follicular maturation in *M. fascicularis* would facilitate ovulation induction and detection studies in this species.

Serial observations of ovarian function were accomplished by laparoscopy, and were begun on the tenth day of the menstrual cycle. Observations were continued over a period of three to four days, at intervals of 4 to 12 hours, depending on the state of the ovary relative to ovulation. As the follicle approached ovulation, observations were made 4 hours apart. In three cases, observations were made every hour for 9 hours, with the animal under continuous anesthesia. These observations were undertaken in the attempt to observe the moment of ovulation.

Behavior

The evaluation of mating behavior in *M. fascicularis* on various days of the menstrual cycle was initiated in order to provide a systematic means of classification which could be used as a basis for selecting both males and females for breeding studies. The following method was devised to quantify the components of reproductive behavior of the macaque in the breeding situation. The Success Ratio

(S.R.) is a modification of Michael's (Michael et al, 1967) and was used to evaluate the relationship of male sexual behavior to ovarian secretory activity. The male Success Ratio was calculated by dividing the number of male initiated mounts (a mount which did not follow a presentation by the female) by the total number of mounting attempts, and multiplying by 100.

$$\text{(Male) S.R.} = \frac{\text{\# male initiated mounts}}{\text{\# male mounting attempts}} \times 100.$$

The female Success Ratio was defined as the number of successful mounting invitations (presentations followed by a mount) divided by the total number of mounting invitations per trial, and multiplied by 100.

$$\text{(Female) S.R.} = \frac{\text{\# successful mounting invitations}}{\text{total mounting invitations/trial}} \times 100.$$

Use of the female S.R. was first described by Michael in 1968 (Michael and Welegalla, 1968); again, in relation to ovarian hormones.

The Reproduction Performance Index (R.P.I.) was developed independently by the author, but is similar in some respects to the Sexual Performance Index (S.P.I.) described by Michael in 1967 (Michael et al., 1967; Michael and Saayman, 1967). Michael's S.P.I. was specifically designed to evaluate the behavior of the male, and defined as the number of mounts to ejaculation, divided by the ejaculation time x 60. The ejaculation time is defined as the

number of minutes from the start of the session to the occurrence of ejaculation. The S.P.I. then is defined as "a measure of male sexual activity obtained from the product of the mounting rate to ejaculation and the mean number of thrusts per mount to ejaculation" (Michael et al., 1967). The S.P.I. is therefore dependent on the detection and timing of ejaculation. Ejaculation was cited by Michael as occurring after a prolonged intromission, during which the male stopped thrusting, arched his back, exhibited spasmodic contractions, and discharged a copious amount of semen.

While Michael used *M. mulatta* in his investigation, the study under consideration here was based on the behavior of *M. fascicularis*, and in this species the arching of the back, spasmodic contractions, and the discharge of semen were not in evidence in all, or even in the majority of cases. Therefore, since the detection of ejaculation was not consistently possible in *M. fascicularis*, the rejection of the Sexual Performance Index was justified in favor of another acceptable standard, the Reproduction Performance Index.

The R.P.I. was the result of an attempt to establish such a standard, and was based on the following information:

Components of the Male R.P.I.

(1) # male initiated mounts
(2) # thrusts per mount ($\bar{X}$ score/total mounts)
(3) vigor of thrusts ($\bar{X}$ thrust/sec x 2)
(4) # female presentations refused.

1. A male initiated mount was one which did not immediately follow a presentation by the female, and which was usually subsequent to the male's grasping of her legs or hips and drawing of the female toward him.
2. The number of thrusts per mount was computed in the R.P.I. on the basis of a scoring system, designed to reflect variations in thrusting behavior while maintaining a proportionate distribution of points amoung the various criteria within the R.P.I.

# Thrusts	Score
0-5	1
6-10	2
11-15	3
16-20	4
21-25	5
>25	6

3. The evaluation of relative vigor of thrusting behavior was a problem until it was observed that the subjective evaluation of thrusting behavior correlated favorably with the number of thrusts per second. It was therefore established that the mean number of thrusts per second multiplied by two would provide a "vigor index" and give this value equal weight in the formulation of the Male R.P.I., i.e., the absolute number, multiplied by 2, would result in a value which would fall within the numerical range of the other components of the index.

4. The number of female presentations refused meant that any presenting behavior, whether it consisted of raising the hips, head-ducking, or other postures which could be considered as mounting invitations, were scored and subtracted quantitatively from the sum of the other factors in the R.P.I.

The Male R.P.I. was therefore computed according to the following:

male initiated mounts _____
thrusts/mount (mean score) _____
vigor of thrusts ($\bar{X}$ thrusts/sec x 2) _____
female presentations refused - _____
Male R.P.I. = _____

The Female R.P.I. was formulated according to the following criteria:

(1) # presentations
(2) receptivity during mount (mean score/total mounts)
(3) # refusals of male attempts to mount.

1. The number of presentations was the total number of times that the female assumed a posture which was considered a mounting invitation.
2. The matter of female receptivity during the mount was more difficult to quantify since it was more of a subjective evaluation. However, there were a number of behavioral components which provided clues concerning the receptivity of the female. The

"receptivity score" was therefore computed according to the following information:

A score of 1 indicated that the female escaped or attempted to escape the grasp of the male during the mount.

A score of 3 was given if the female remained passive throughout the mount.

A score of 5 was awarded if the female reached back toward the male and grasped or attempted to grasp his leg, foot, or scrotum. These activities were interpreted as attempts to retain the male in the mounting position.

3. The number of refusals of male attempts to mount is self-explanatory, and was easy to observe. The total number of male refusals was subtracted from the sum of the other factors in the Female R.P.I.

The Female R.P.I. was therefore computed according to the following:

\# presentations _____
receptivity during mount (mean score) _____
\# refusals of male attempts to mount - _____
Female R.P.I. = _____

Examples of R.P.I. and S.R. data recording sheets, with the corresponding computations for one mating trial are included in Appendix II.

All mating sessions were conducted for 30 minutes in a special cage unit equipped with a one-way glass, and isolated from the rest of the colony.

RESULTS

The data indicated in the following text and in the tables will be reported as mean values ± the standard deviation.

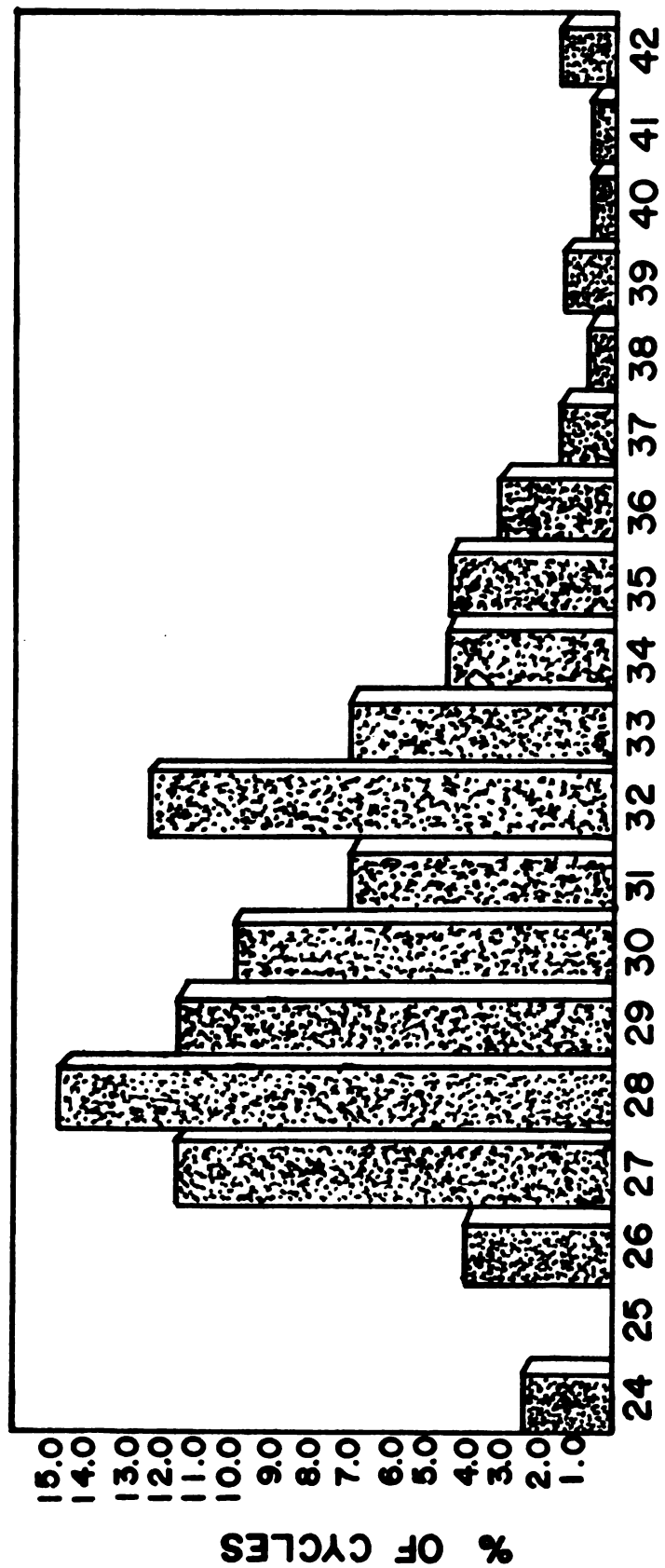
Reproductive Parameters

The distribution of menstrual cycle lengths is illustrated in Figure 5. In the females observed for a period of 8 months, the mean cycle length for 129 cycles was 30.8 ± 4.6 days; the median, 33.0 days; and the mode, 28.0 days (14.7% of all cycles). The percentage of cycles ranging from 27 to 32 days in length was 66.4%. Cycles ranged from 24 to 42 days, and cycles of longer than 45 days were excluded on the basis that such cycles were reported in animals where the presence of menstrual flow was questionable, or where the presence of blood in a cage was attributable to trauma or disease.

Since the method of observing menses introduces an unavoidable source of error in the establishment of the first day of the menstrual cycle, regular cycles were scored on the basis of menstrual flow beginning within 2 days of the expected date, based on an average length of previous menstrual cycles. Animals which would be included by this

FIGURE 5

DURATION OF THE MENSTRUAL CYCLE IN MACACA FASCICULARIS



5 day bracket were considered acceptable for further study. A comparison of regular and irregular cycles is expressed in Table 3. Of the 141 cycles observed, 88 (62.4%) were within 2 days of the projected duration, as compared to 53 (37.6%) which were irregular.

Table 3. Comparison of regular and irregular menstrual cycles in *M. fascicularis*

	% of Total (141)	$\bar{X}$ Length (days)
Regular	62.4% (88)	29.2 ± 3.7
Irregular	37.6% (53)	33.1 ± 5.1

The mean cycle length for animals with regular cycles was 29.2 ± 3.7 days, whereas the mean for those with irregular cycles was considerably longer, 33.1 ± 5.1 days. Of all animals, 72.3% had regular cycles 50% of the time or more; 48.2%, 60% of the time or more; and 44.8% had 70% or more regular cycles (Table 4).

Table 4. Percent regular cycles for individual animals (percent having regular cycles less than 30% of time, 30-39% of time, etc.)

<30%	30-39%	40-49%	50-59%	60-69%	70% +
20.7% (6/29)	3.4% (1/29)	3.4% (1/29)	24.1% (7/29)	3.4% (1/29)	44.8% (13/29)

In a comparison of the duration of follicular and luteal phases (Table 5), where ovulation was determined by laparoscopy, the mean length of the follicular phase was 15.4 ± 3.0 days; and the mean length of the luteal phase was 13.6 ± 2.5 days. The mean cycle length for the 5 cases was 29.0 ± 3.5 days. Two of the five observations were made in the same monkey (#52), and the percentage of the cycle length attributed to follicular and luteal phases in each cycle was 54.3% (follicular) and 45.7% (luteal) in the first cycle; and 61.6% (follicular) and 38.4% (luteal) in the second cycle. This compares with mean percentages of 53.2% for the total of 5 follicular phases, and 46.8% for the 5 luteal phases.

The duration of menstrual flow in 150 cycles observed is expressed in Figure 6. The mean number of days of menstrual flow was 2.8 ± 1.4 days; the median, 4 days, and the mode, 2 days. Menses ranged from 1 to 8 days in length, with 90.7% of cases reported ranging from 1 to 4 days in length.

In Figure 7, the relationship between the number of days of light, moderate, and heavy flow, and various cycle lengths is illustrated. One of the obvious patterns which emerges is the declining percentage of observations on day 1 which are classified as light. The converse of this pattern is also evident, in that the percentage of cases with heavy flow on day 1 increases as the number of days of menses increases. For a given duration of menses, the percentage

Table 5. Relationship of follicular and luteal phases to the length of the menstrual cycle in *M. Fascicularis*

Female #	Time of laparos-copy before ovulation	Time of laparos-copy after ovulation	Time of ovulation	Onset of menses	Cycle length (days)	Follic-ular phase (days)	(% of cycle)	Luteal phase (days)	(% of cycle)
8	4 PM 7/22	9 AM 7/23	10 PM 7/22	10 PM 8/3	28	15	53.7	13	46.3
44	10 AM 9/20	2 PM 9/21	12 AM 9/21	12 AM 10/4	28	15	53.7	13	46.3
55	11:30 AM 10/6	11 AM 10/7	12 AM 10/7	12 AM 10/23	28	12	42.9	16	57.1
52	10:35 AM 12/10	12:30 PM 12/11	12 AM 12/10	12 AM 12/27	35	19	54.3	16	45.7
52	11:45 PM 1/11	2:30 PM 1/12	12 AM 1/12	12 AM 1/21	26	16	61.6	10	38.4
					$\bar{X}$ = 29	15.4	53.2	13.6	46.8
					S.D. ± 3.5	± 3.0		± 2.5	

FIGURE 6

DURATION OF MENSTRUAL FLOW IN
MACACA FASCICULARIS

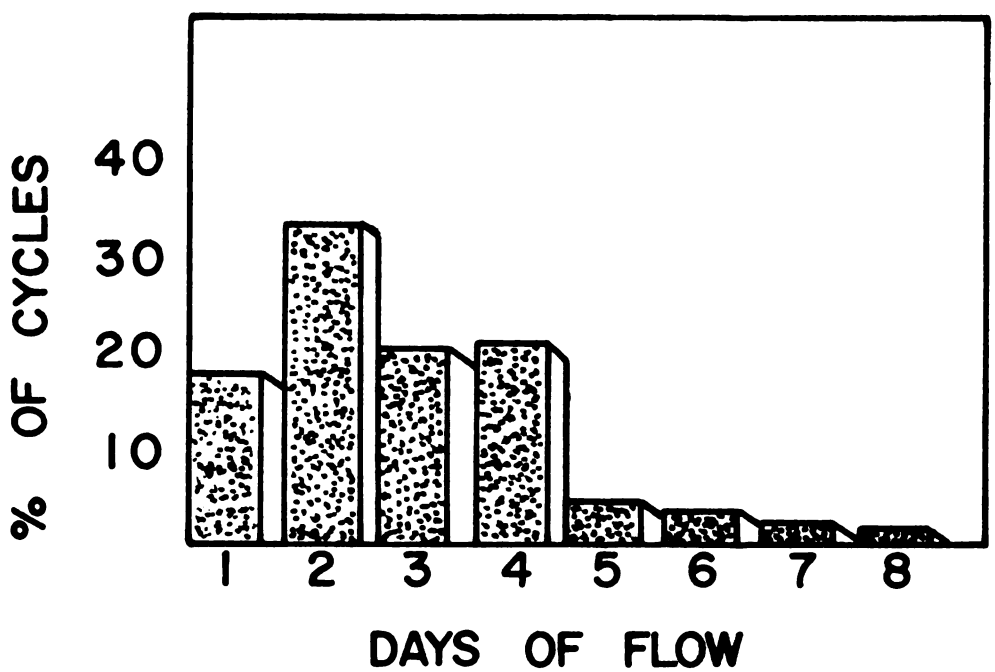
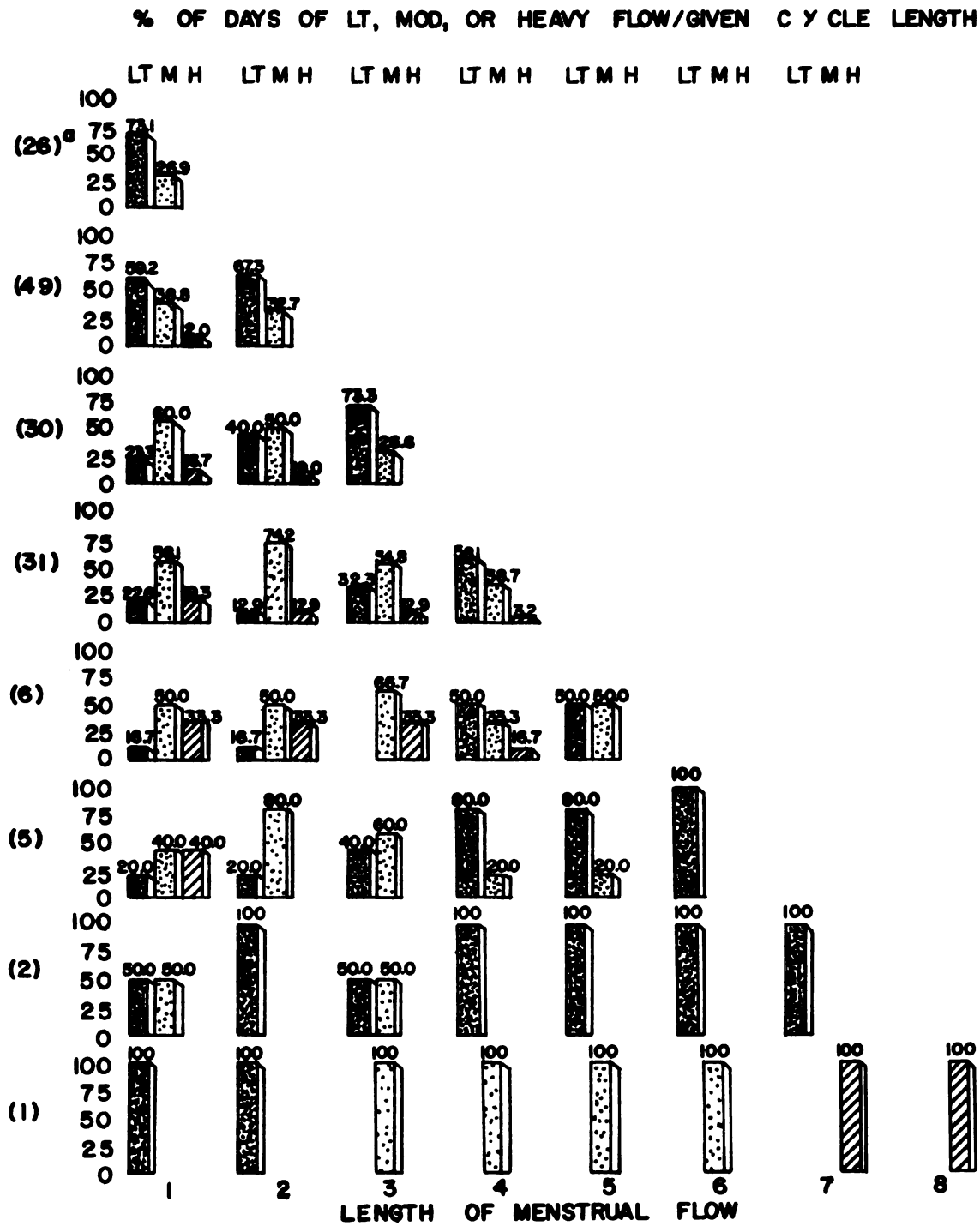


FIGURE 7



(a) # observations in parenthesis

cases of light flow increased as the end of menstrual flow was reached. The total number of days of light flow was 200 (47.6%), moderate flow, 185 days (44.1%), and heavy flow, 35 days (8.3%). The distribution of cycles according to the number of days of menstrual flow is indicated in Table 6.

Table 6. Relationship of menstrual cycles to number of days of menstrual flow in *M. fascicularis*

Days of flow	# Cycles	% of Total
1	26	17.3
2	49	32.7
3	30	20.0
4	31	20.7
5	6	4.0
6	5	3.3
7	2	1.3
8	1	0.7

The incidence of pregnancy according to the day of the menstrual cycle on which mating occurred is indicated in Table 7. In the animals with average cycle lengths of 28 days, conception occurred on days 11 and 12 of the cycle; whereas, in animals with 32 day cycles, pregnancy was achieved on days 14 and 15 of the cycle. Assuming that ovulation occurred on the day of successful mating, all four

Table 7. Relationship of day of cycle to successful mating in *M. fascicularis*

Cycle day of successful mating	Ave. cycle length[a]
14	32
11	28
12	28
15	32
$\bar{X}$ = 13 ± 1.8	30 ± 2.3

[a]Average based on minimum of six previous cycles.

pregnancies occurred between 16 and 18 days before the expected initiation of the next menses.

The gestation lengths and associated implantation bleeding for the three pregnancies which were permitted to go to term are indicated in Table 8.

Table 8. Gestation length and implantation bleeding in *M. fascicularis*

Female #	Gestation length[a] (days)	Duration of implantation bleeding (days)	Inception of flow (day of pregnancy)
54	164.6	9	18
42	165.4	9	18
39	163.1	4	21
$\bar{X}$	164.5	7.3	19.0

[a]Based on average of shortest and longest estimates; e.g., it was known that female # 54 was mated at a specific time, but the delivery occurred between two observations, made six hours apart. The gestation length was therefore calculated from mating until three hours before the last observation.

Ovulation Induction and Follicular Aspiration

In order to evaluate the follicular aspiration device the PMS/HCG regime previously described was introduced. Each of the animals used in this study had been laparoscoped prior to the induction trial to provide a comparison for post-induction observations. The results are shown in Table 9, as observed by laparoscopy on the day following the last injection of HCG. All of the follicles were aspirated and the contents examined for the presence of ova. Considerable difficulty was encountered due to the presence of excessive amounts of ovarian

Table 9. Results of PMS/HCG superovulation regime in *M. fascicularis*

Animal #	Right ovary	Left ovary
10	2 characteristic pre-ovulatory follicles; several cystic protrusions.	Unable to manipulate for observation.
38	4 cystic follicles, 3 x size of normal.	5 follicles similar in appearance to those on right.
26	1 normal pre-ovulatory follicle. Ovary 2 x normal size. Total normal follicles = 5 Total cystic follicles = 9	2 normal pre-ovulatory follicles. Ovary 2 x normal size

tissue and erythrocytes, and no ova were found. A total of 1.5 ml of follicular fluid was aspirated.

Temperature Variation in the Menstrual Cycle of *M. fascicularis*

Rectal temperature recordings were made of monkeys which had been removed from their cages and manually restrained. The first objective of this study was to determine if the rectal temperature showed fluctuations which may be associated with ovulation. The temperature records for the 4 cycles observed are illustrated in Figure 8. The means for each cycle are listed in Table 10. This study was discontinued on the basis that the manipulation of the animals for temperature recordings resulted in individual

FIGURE 8

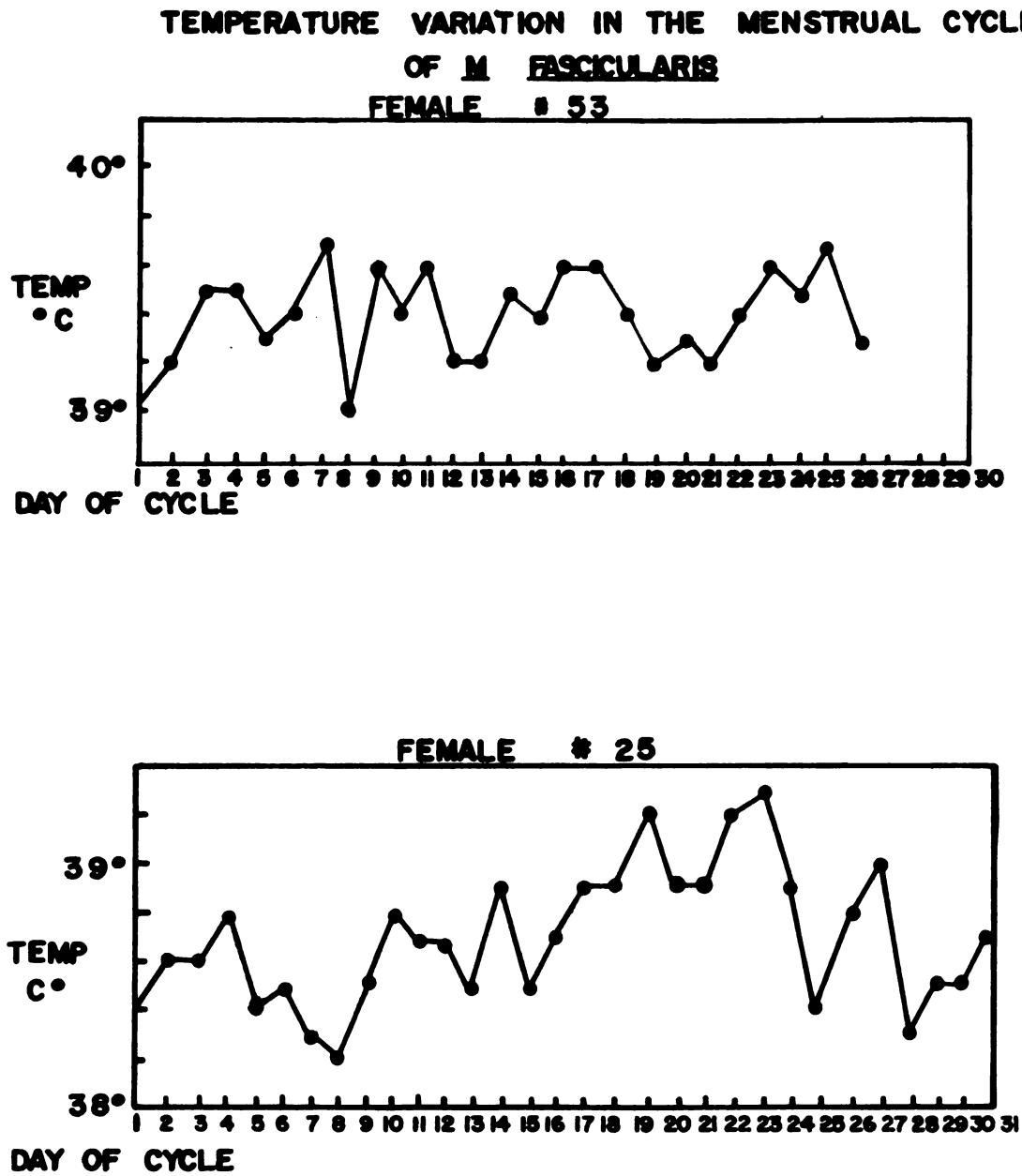
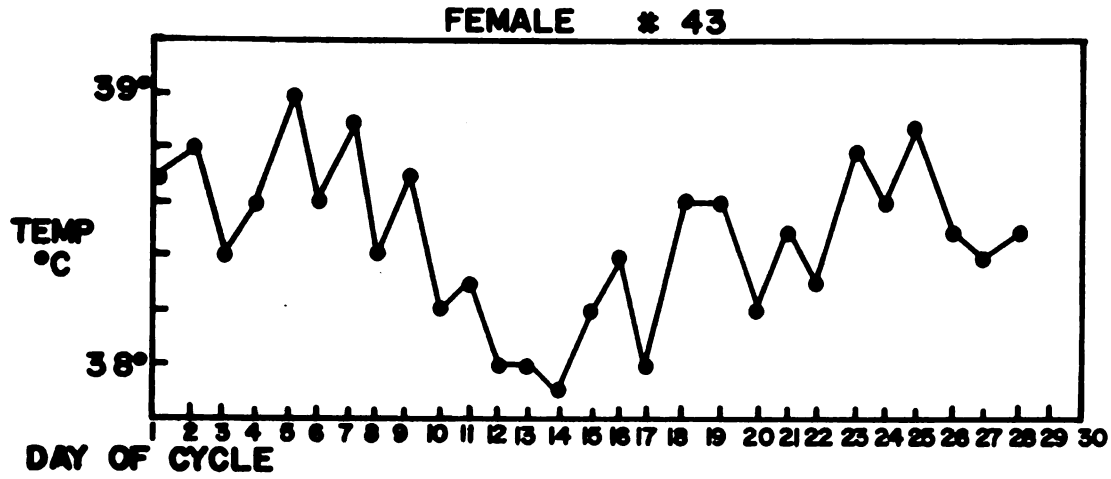

TEMPERATURE VARIATION IN THE MENSTRUAL CYCLE
OF M FASCICULARIS
FEMALE # 53
40°
TEMP
°C
39°
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
DAY OF CYCLE
FEMALE # 25
39°
TEMP
C°
38°
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
DAY OF CYCLE

FIGURE 8 Con't.



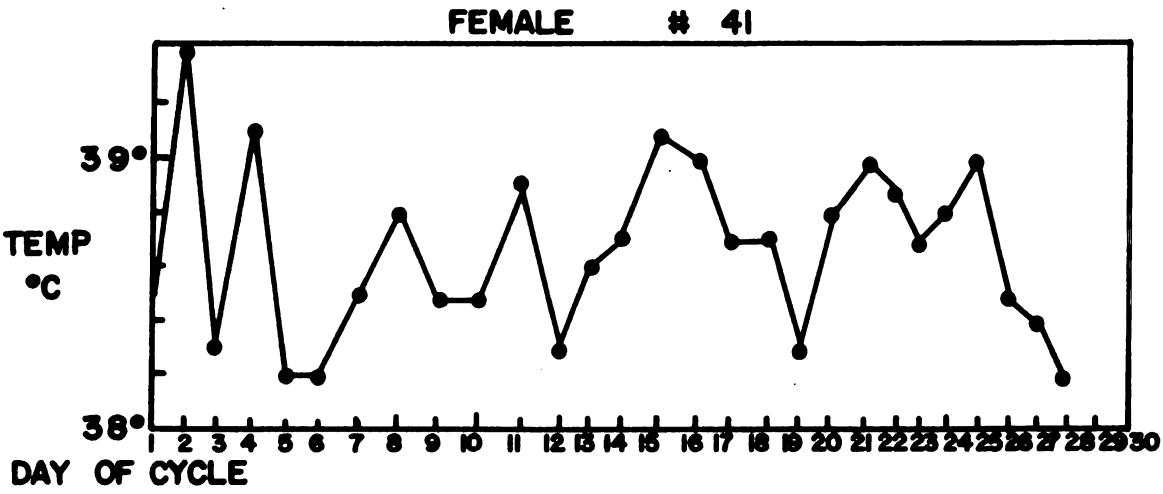


Table 10. Temperature variation in the menstrual cycle of *M. fascicularis*

Female #	$\bar{X}$ temp./cycle (°C)	Range
53	39.4 ± 0.2	39.0-39.7
25	38.7 ± 0.3	38.2-39.3
43	38.5 ± 0.3	37.9-39.0
41	38.7 ± 0.3	38.2-39.4
$\bar{X}$ of all cycles =	38.8 ± 0.4	
	Range of all cycles =	37.7-39.7

variations (0.7°C to 1.3°C) which precluded the detection of temperature fluctuations which might be associated with ovulation.

Behavior

In the evaluation of reproductive behavior in short duration matings (30 minutes per trial), it was found that the Female Reproduction Performance Index (R.P.I.) and Success Ratio (S.R.) showed greater variation according to the day of the cycle on which mating occurred than did the Male R.P.I. and S.R. This relationship can be seen by comparing Figures 9 and 10. When these figures are broken down according to individual cycle lengths (Figures 11, 12, 13), again, the female scores exhibited the greatest variation. For the Female R.P.I. scores, the highest values were achieved on day 11 of the 28 day cycles, day 14 of the 30 day cycles, and day 16 of the 32 day cycles. Inspection of female S.R. scores shows that the highest scores were found

FIGURE 9

RELATIONSHIP OF FEMALE RPI & SR TO MATINGS
ON VARIOUS DAYS OF THE MENSTRUAL CYCLE

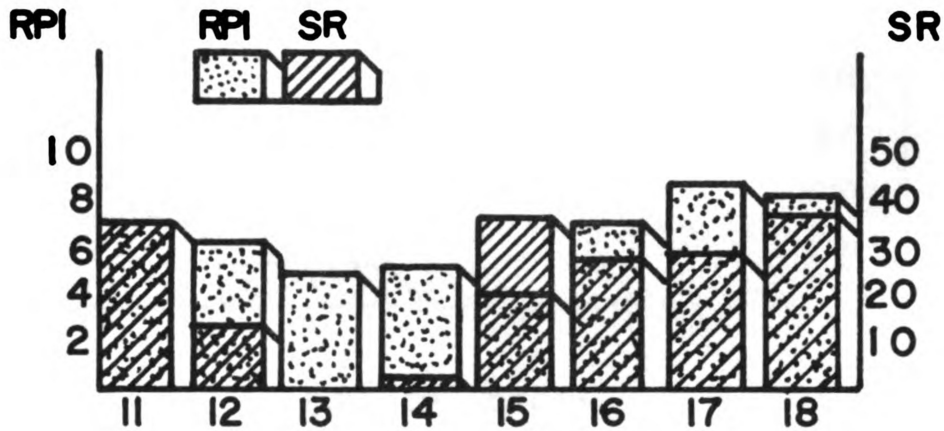


FIGURE 10

RELATIONSHIP OF MALE RPI & SR TO MATINGS
ON VARIOUS DAYS OF THE MENSTRUAL CYCLE

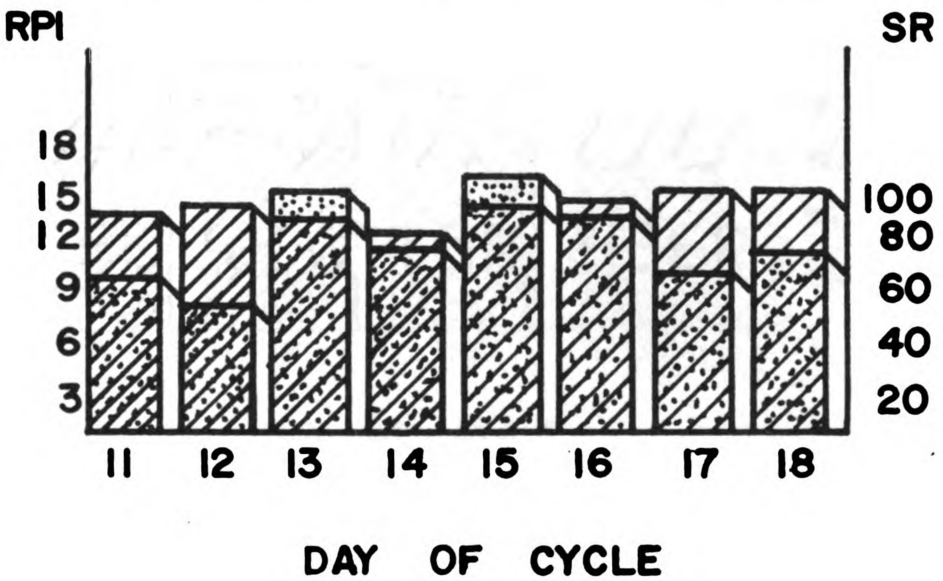
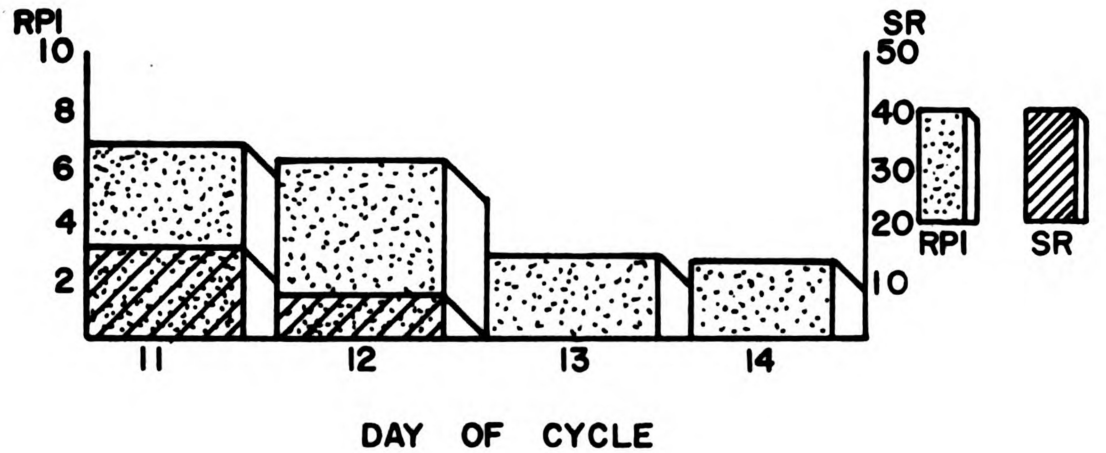


FIGURE 11

RELATIONSHIP OF FEMALE RPI & SR TO MATINGS
ON DAYS 11 THROUGH 14 OF 28 DAY CYCLE



RELATIONSHIP OF MALE RPI & SR TO MATINGS
ON DAYS 11 THROUGH 14 OF 28 DAY CYCLE

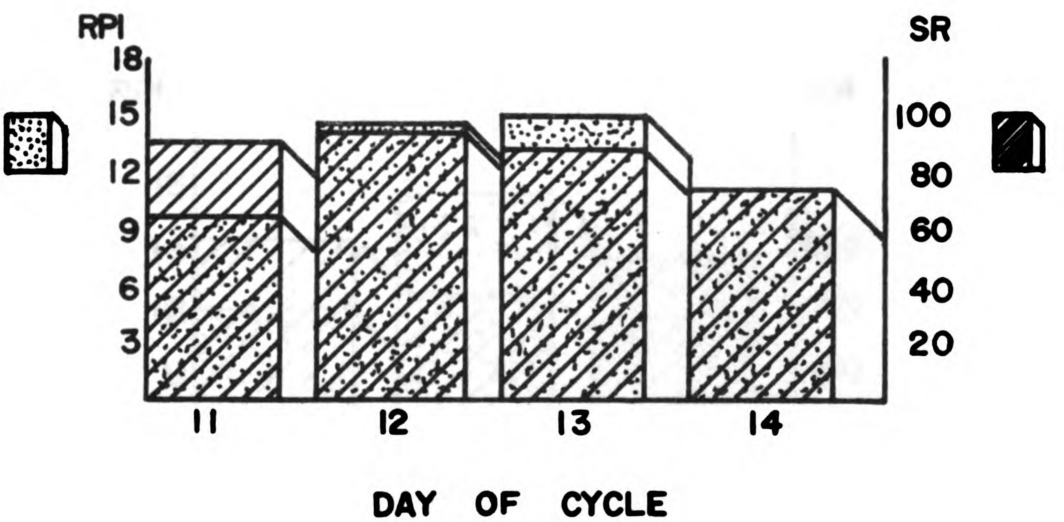
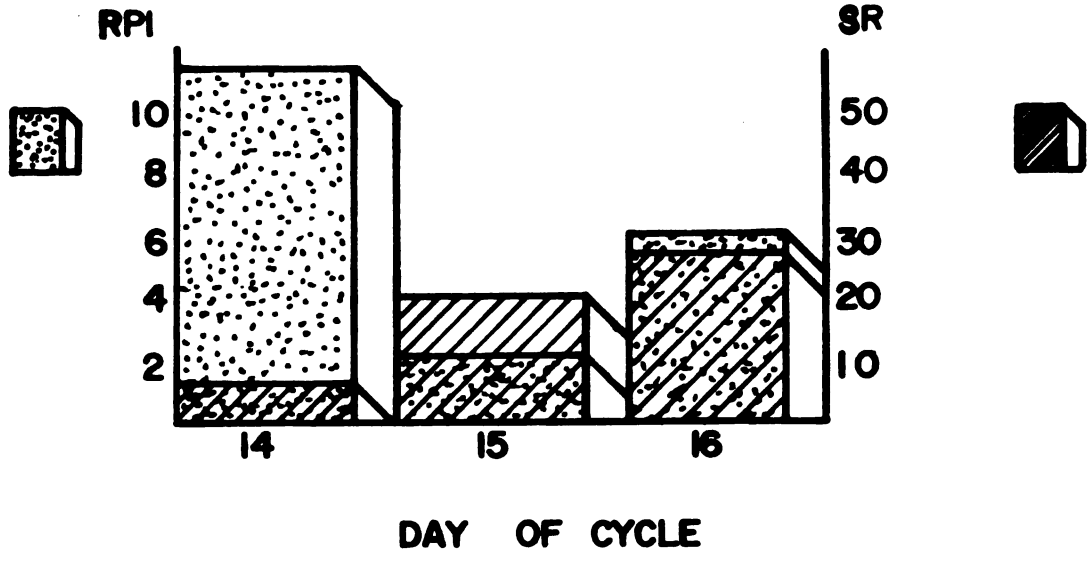


FIGURE 12

RELATIONSHIP OF FEMALE RPI & SR TO MATINGS
ON DAYS 14 THROUGH 16 OF 30 DAY CYCLE



RELATIONSHIP OF MALE RPI & SR TO MATINGS
ON DAYS 14 THROUGH 16 OF 30 DAY CYCLE

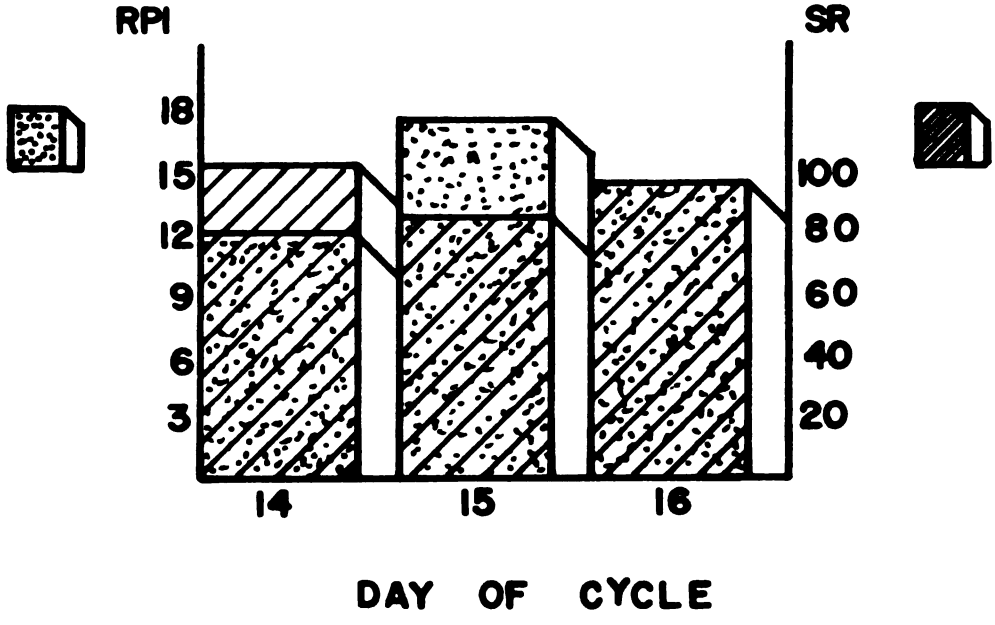
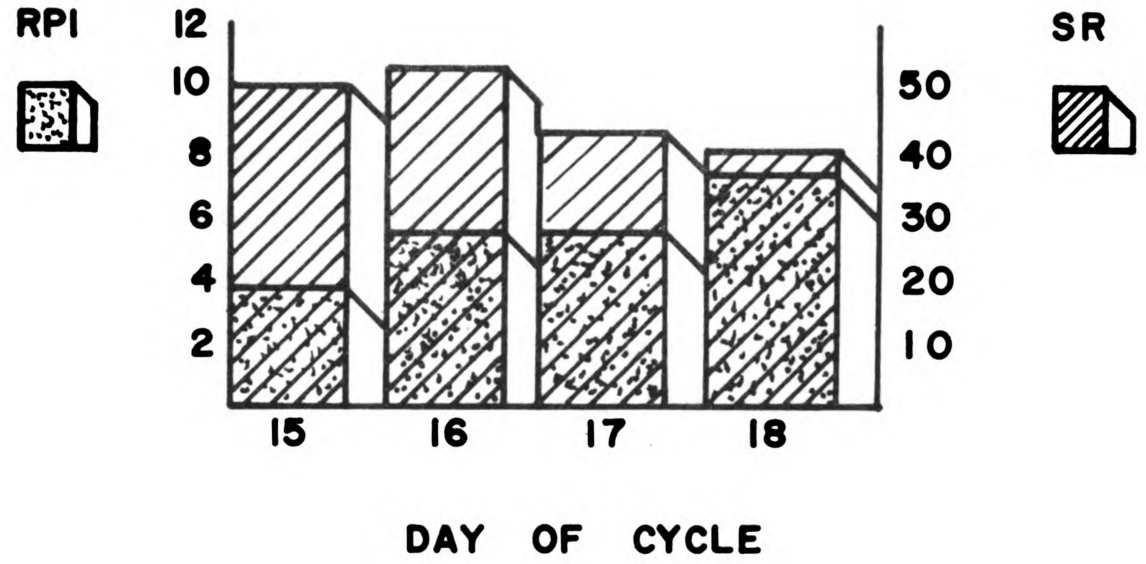
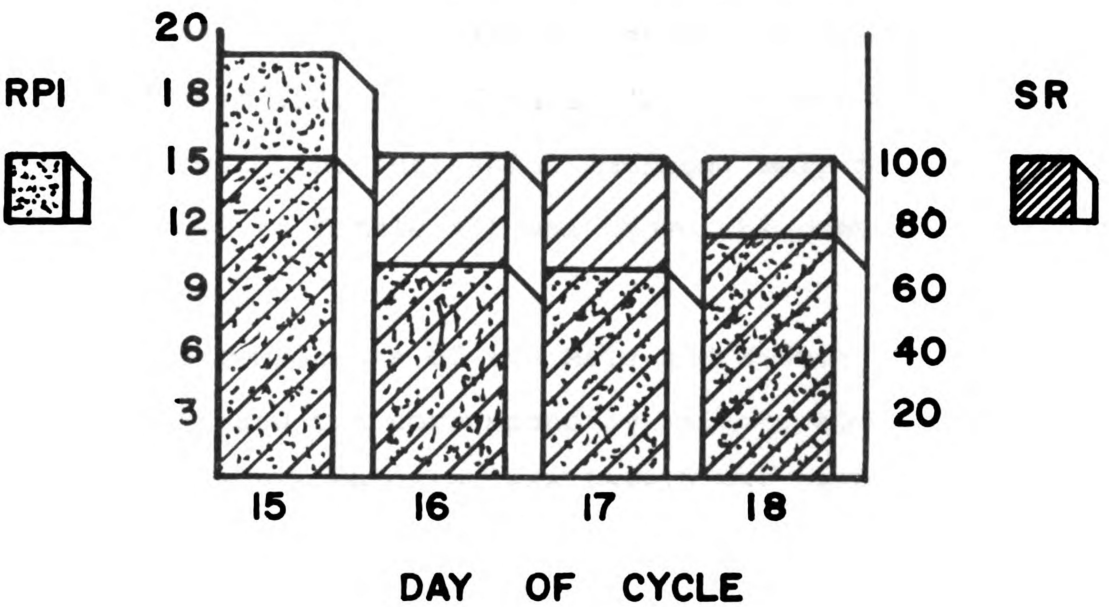


FIGURE 13

RELATIONSHIP OF FEMALE RPI & SR TO MATINGS
ON DAYS 15 THROUGH 18 OF 32 DAY CYCLE



RELATIONSHIP OF MALE RPI & SR TO MATINGS
ON DAYS 15 THROUGH 18 OF 32 DAY CYCLE



on day 11 of the 28 day cycles, day 16 of the 30 day cycles, and day 15 of the 32 day cycles. The mean scores for both Male and Female R.P.I. and S.R., as well as the number of observations in each category are listed in Tables 11 and 12.

For the successful matings (those resulting in pregnancy) which occurred while the behavior scoring system was in effect, female #7 had an R.P.I. of 15.4 and S.R. of 41.7; while her male partner had an R.P.I. of 9.2, and a S.R. of 100. For female #42, the R.P.I. score was 9.0, while the S.R. was 14.0; with her male partner scoring an R.P.I. of 10.0, and a S.R. of 88.9. The mean Female R.P.I. for all observations was 5.6, the S.R., 25.6. The overall mean for the Male R.P.I. was 12.7; and for the S.R., 90.3. In each of the successful matings reported, the Female R.P.I. scores were above the mean for all observations (5.6), while one of the S.R. scores (41.7 for female #7) was above the mean for all observations (25.6), and the other was considerably below the mean (14.0 for female #42). Of the male scores associated with the successful matings, only one value (S.R. of male mated to female #7) was above the mean for all observations.

The components of the Male and Female R.P.I. and S.R. scores, and the corresponding values relating to the days of the cycle on which the female was mated, are provided in Table 13. The highest number of male mounting attempts and female presentations occurred on day 12 of the cycles, and the peak values for male initiated mounts and

Table 11. Female R.P.I. and S.R. according to cycle length and day of cycle mated

Day of cycle	Cycle length				
	28	29	30	32	$\bar{X}$
	R.P.I./S.R. (# observations)				
11	(4) 6.8/16.7				(4) 6.8/16.7
12	(2) 6.15/7.0				(2) 6.15/7.0
13	(3) 2.9/0.0				(3) 2.9/0.0
14	(7) 2.7/0.0		(2) 11.6/6.2		(9) 4.7/1.4
15		(1) 8.0/4.0	(2) 2.1/20.0	(2) 3.8/50.0	(5) 4.0/36.0
16			(3) 6.0/27.8	(1) 10.7/28.0	(4) 7.2/27.8
17				(2) 8.5/28.5	(2) 8.5/28.5
18				(2) 8.0/35.7	(2) 8.0/35.7

Table 12. Male R.P.I. and S.R. according to cycle length of female and day of cycle mated

Day of cycle	Cycle length				
	28	29	30	32	$\bar{X}$
			R.P.I./S.R. (# observations)		
11	(4) 9.8/91.6				(4) 9.8/91.6
12	(2) 14.8/94.4				(2) 14.8/94.4
13	(3) 15/87.5				(3) 15/87.5
14	(7) 11.2/75.6		(2) 12/100		(9) 11.4/81
15		(1) 9.6/100	(2) 17.4/83	(2) 19.4/100	(5) 16.6/93.2
16			(3) 14.4/95.2	(1) 9.8/100	(4) 13.2/96.4
17				(2) 9.8/100	(2) 9.8/100
18				(2) 11.5/100	(20 11.5/100

Table 13. $\overline{X}$ component scores of Male and Female R.P.I. and S.R.

	Day of cycle							
	11	12	13	14	15	16	17	18
Behavior Parameters (Male):								
Male initiated mounting attermpts/ trial	4.3	9.7	7.0	5.1	9.4	7.1	4.0	4.5
Male initiated mounts/trial	3.7	9.0	6.0	3.8	8.2	7.0	4.0	4.5
Thrusts/mount	3.9	4.2	4.5	4.3	4.6	4.5	4.2	5.2
Vigor of thrusts	2.8	3.0	3.7	3.5	3.1	3.4	4.0	4.8
Thrusts/second	3.3	3.7	5.2	3.1	4.0	3.9	4.0	4.8
Presentations refused	1.0	4.3	3.8	0.5	1.2	3.0	2.5	3.0
Behavior Parameters (Female):								
Presentations	1.3	6.3	3.5	0.5	2.2	4.3	4.5	4.0
Successful presentations	0.3	2.0	0.5	0.0	1.0	1.3	2.0	2.5
Receptivity during mount	3.0	3.2	3.0	3.5	3.0	3.7	4.0	4.0
Refusals of male attempts to mount	0.3	0.3	0.5	1.1	1.2	0.3	0.0	0.0

mounting attempts occurred on days 12 and 15 of the menstrual cycle. The highest number of female presentations refused, as well as the highest number of presentations, occurred on the 12th day of the cycle.

Follicular Morphology

Using the technique of serial laparoscopy in *M. fascicularis*, it was found that the anatomical changes associated with pre- and post-ovulatory follicular development could be observed (Plates 1 and 2). The site of the developing follicle could be identified 2 days prior to ovulation by (a) a generalized swelling and darkening of a portion of the ovarian surface; (b) an increase of approximately 35% in the size of the ovary; and (c) a more uniform surface texture than was apparent on the ovary which did not contain the pre-ovulatory follicle (Plate 1, Figures 1, 2).

Within 30 hours of ovulation, a stellate pattern of blood vessels was observed on a portion of the ovary which would eventually form the follicular wall (Plate 1, Figures 3, 4). Within 8 to 10 hours of ovulation, the pattern of vessels became more pronounced, and the follicular cone was established (Plate 2, Figure 4). In 80% of the cases observed, this pattern presented a network of smaller vessels around the base of the follicular cone, and a larger vessel across the center of the follicular wall which bifurcated at the follicular border (Plate 2, Figures 4, 5; Plate 3). The vessel across the center of the follicle

PLATE 1

PRE-OVULATORY FOLLICULAR MORPHOLOGY IN *M. FASCICULARIS*.
Days 10, 11 of menstrual cycle.

Fig. 1. Day 10 of cycle. Arrow marks site of developing follicle on left ovary. Note difference in color and shape of right ovary.

Fig. 2. Day 10 of cycle. Same stage of follicular development as on right ovary in Fig. 1, but in another monkey. Note grey area at arrow indicating site of follicle.

Fig. 3. Day 11 of cycle. Stellate pattern of blood vessels established on follicular wall. A indicates vessel which will be across center of follicle as development proceeds. B indicates tissue which will form follicular hemispheres.

PLATE 1

Fig. 1

Fig. 2

Fig. 3

PLATE 2

PRE-OVULATORY FOLLICULAR MORPHOLOGY IN *M. FASCICULARIS*.
Day 12 of menstrual cycle.

Fig. 4. Day 12 of cycle. Follicular cone is established. Note vessel at center of follicle A; and bifurcation at edge of follicle.

Fig. 5. Day 12, 4 hours after Fig. 4. Note formation of stigmata (arrows), and enlargement of left hemisphere.

Fig. 6. Day 12, 2 hours after Fig. 5. Fimbria covering enlarged hemisphere and stigma.

Plate 2

Fig. 4

Fig. 5

Fig. 6

PLATE 3

POST-OVULATORY FOLLICULAR MORPHOLOGY IN *M. FASCICULARIS*.
Days 14, 15 of menstrual cycle.

Fig. 7. Post-ovulatory follicle of day 14 on right, compared to pre-ovulatory follicle of day 12. Note hemorrhagic appearance and loss of definition of follicular vessels in post-ovulatory follicle.

Fig. 8. Day 14 of cycle. Note irregularity of follicular wall (arrow) where the wall is partially collapsed.

Fig. 9. Day 15 of cycle. Follicle still hemorrhagic, some luteinization visible.

PLATE 3

Fig. 7

Fig. 8

Fig. 9

divided it into two discrete hemispheres, each displaying a localized area of avascular tissue which appeared to be stigmata (Plate 1, Figure 5; Plate 3). In several cases, the fimbria was observed in contact with one of the follicular hemispheres (Plate 1, Figure 6).

A vascular pattern found in approximately 20% of the cases observed was characteristic of follicles of the single hemisphere type (Plate 6, Figure 1), where a single stigma was found at the bifurcation of the major follicular vessel.

The pre-ovulatory follicle in *M. fascicularis* is compared to the smaller pre-ovulatory follicular cone found in *Saimiri sciureus* (Plate 6, Figure 2).

The most significant post-ovulatory changes were found to be (a) a generalized hemorrhagic appearance (Plate 2, Figure 7); (b) a loss of definition of the follicular vasculature (Plate 2, Figures 7, 8, 9); (c) the progressive occlusion of the stigmata (Plate 2, Figure 10); (d) a flattening, or irregularity of the follicular wall (Plate 2, Figure 8); and (e) partial luteinization (Plate 2, Figures 9, 10, 11). These characteristics could, in some cases, be observed as early as 10 hours following ovulation, but the corpus hemorrhagicum (CH) generally could not be reliably distinguished from the pre-ovulatory follicle until 20 to 40 hours following ovulation.

PLATE 4

POST-OVULATORY FOLLICULAR MORPHOLOGY IN *M. FASCICULARIS*.
Days 16, 20, and 24 of menstrual cycle.

Fig. 10. Day 16 of cycle. Corpus luteum is assuming nodular appearance. Occlusion of stigmata is nearly complete.

Fig. 11. Day 20 of cycle. Further luteinization of CL. Vascular pattern has begun to recede.

Fig. 12. Day 24 of cycle. Note, follicular vessels may persist through the end of a given cycle.

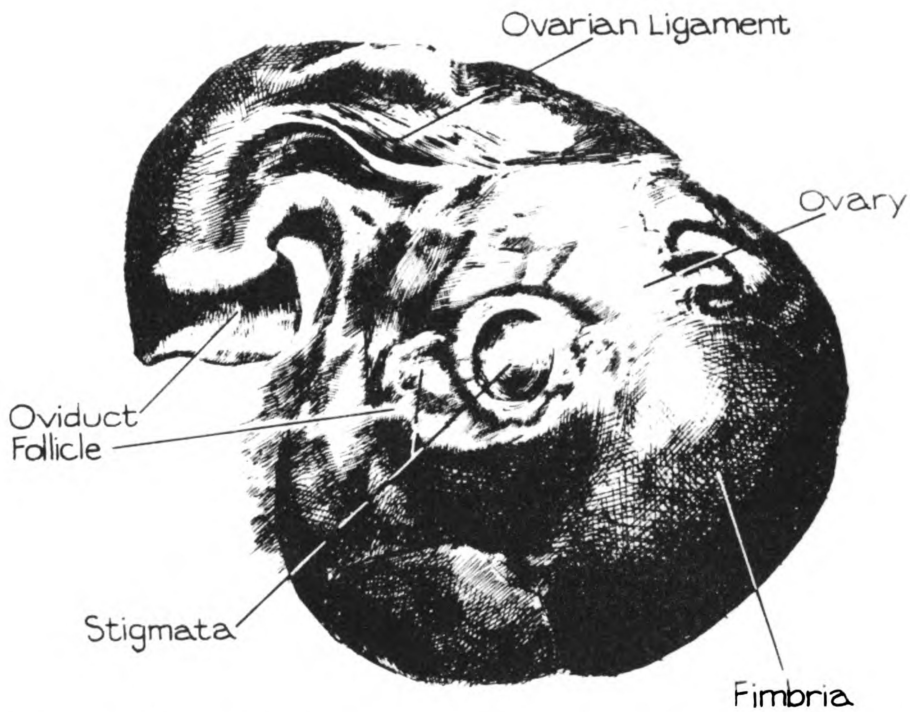
PLATE 4

Fig. 10

Fig. 11

Fig. 12

PLATE 5



PRE-OVULATORY FOLLICLE, LESS THAN 10 HOURS BEFORE OVULATION

PLATE 6

INFRA-RED PHOTOGRAPHY THROUGH THE LAPAROSCOPE

Fig. 1. Uterus of *M. fascicularis* as photographed through the laparoscope with standard A and infra-red B color film. Note greater detail in upper and lower right of the infra-red photography.

Fig. 2. Infra-red photograph of ovary containing early follicle in *M. fascicularis*. Blue color indicates area of follicle.

Fig. 3. Pre-ovulatory follicle of single hemisphere type; uterus on right (*M. fascicularis*).

PLATE 6

Fig. 1

Fig. 2

Fig. 3

PLATE 7

VARIABLE APPEARANCE OF CORPORA HEMORRHAGICA

Figs. 1, 2. Corpora hemorrhagica from 2 different monkeys *(M. fascicularis)* on day 16 of the cycle, illustrating variability of appearance of CH.

PLATE 8

SINGLE HEMISPHERE FOLLICLE IN *M. FASCICULARIS* AND PRE-OVULATORY FOLLICLE IN *S. SCIUREUS*

Fig. 1. Pre-ovulatory follicle in *M. fascicularis* of single hemisphere type. Note stigma (arrow) at point of bifurcation of blood vessel.

Fig. 2. Pre-ovulatory follicle in *Saimiri sciureus*. Ovaries at A, and pre-ovulatory follicle at B.

The corpora hemorrhagica in Plate 2, Figure 9, and Plate 5, Figures 1 and 2, emphasize the variable appearance of post-ovulatory follicular changes in *M. fascicularis*. In the majority of cases, the follicular vasculature remained intact through the remainder of the cycle (Plate 2, Figures 11, 12), but, as Plate 2, Figures 7, 8, and 9, and Plate 5 point out, the appearance of the CH is considerably more variable than the pre-ovulatory follicle.

Plate 4 illustrates the results of infra-red photography of the uterus and ovarian follicles in *M. fascicularis*. Figure 1 demonstrates the effectiveness of this technique, compared to standard color photography, when attempting to detect and photograph minute vasculature. Figure 2 shows how infra-red can be used to outline the portion of the ovary which contains the early developing follicle; and Figure 3 points out the detail of the vasculature associated with a follicle of the single hemisphere type.

DISCUSSION

Cyclicity in *M. fascicularis*

The values given by Macdonald (1971) for mean cycle length in *M. fascicularis* (31.3 ± 1.5 days) correlate with the value of 30.8 ± 4.6 days found in the present study. These findings also confirm the findings of Corner (1932) and Macdonald (1971) which suggested that the cycle length of *M. mulatta* is shorter than that of *M. fascicularis* by at least 2 days.

The recording of menstrual cycles and calculation of the next expected onset of menses was essential to studies which were concerned with ovulation. It was first of all necessary to eliminate from these studies animals in which the ovulation time could not be predicted within a range of 2 days. Secondly, we wished to determine if this species was useful as a model for human reproduction studies. It was therefore necessary to demonstrate the variability of menstrual cycles in *M. fascicularis* so that correlations to human cyclicity could be made. Of the 141 menstrual cycles recorded, 62.4% were regular; and, more significantly, 44.8% of the animals were observed to have regular cycles at least 70% of the time. These figures indicated a higher percentage

of regular cycles than has been reported in humans by Brayer et al. (1969), who found that only 30% of 2,316 women who reported 30,655 menstrual cycles had a range of 8 days or less. The range was defined as the difference between the longest and shortest cycles.

The duration of menstrual bleeding was found to have a mean of 2.8 days, which confirms the value of 2.6 days reported by Kerber and Reese (1968). A recent report by Macdonald (1971) indicated a mean flow of 4.2 days. However, Macdonald's study utilized a vaginal smear technique to detect menses. This technique would be expected to identify very slight menstrual flow of the type which would not be recorded by the method used in the present study, thereby resulting in a larger number of days of menstrual flow, and accounting for a larger mean value.

In reproduction studies, one of the primary concerns is the problem of infertility. To consider one aspect of this problem, the relationship of the follicular and luteal phases to the length of the menstrual cycle was investigated in *M. fascicularis*. The follicular phase in four of the five observations was longer than the luteal phase by 2 to 6 days. This suggests that perhaps ovulation does not occur at mid-cycle, but rather several days later. Secondly, as the length of the cycle increased, the length of both follicular and luteal phases increased. This may indicate that the length of the luteal phase may not be as constant in this species as has been reported in *M. mulatta* by Hartman

(1933) and Monroe (1970). Although the length of the follicular phase was found to be slightly more variable than the luteal phase in this study, the difference was not statistically significant. The difficulties encountered in this kind of study are considerable due to the number of laparoscopic examinations required to confirm ovulation within a narrow time bracket. However, the present results are at least interesting grounds for speculation, and will serve as a basis for further investigations in this area.

In a related study, females were mated on days 11 through 18 of the menstrual cycle. Of the four ensuing pregnancies in which the average cycle length was known (Table 4), all animals conceived between 16 and 18 days before the next expected menstruation, although matings had occurred on days 11 to 15 of the menstrual cycles. Since the successful matings on days 11 and 12 of the cycle were with females having 28 day cycles, and the matings on days 14 and 15 were those having 32 day cycles, this would indicate that the optimum time for mating may vary according to cycle length, and that a mating time projected by counting "backward" from the expected end of the menstrual cycle may result in an increased number of pregnancies. This concept closely parallels the scheme of Valerio (1970) who arranged a mating schedule for *M. mulatta* based on differences in cycle length.

It is worth noting that the pregnancies reported in the current study were diagnosed by laparoscopy. Laparoscopic examinations were performed between days 30 and 60 of pregnancy without deleterious effect. During these observations, the uterus was observed to be markedly distended and the ovaries were elongated with the ovarian ligaments clearly visible, stretched across the uterine body. These examinations comprise the first report of pregnancy diagnosis by laparoscopy in the nonhuman primate.

Gestation Length and Implantation Bleeding

The gestation lengths in the four pregnancies observed ranged from 163.1 days to 165.4 days with a mean of 164.5 days. This is shorter than the 167 days reported by Spiegel (1954), 168 days reported by Fujiwara and Imamichi (1966), and 165.7 days reported by Macdonald (1971). The technique of limited duration matings permitted a more accurate estimation of gestation length than was previously obtainable.

The implantation bleeding associated with three of the above pregnancies began on days 18 and 21 of pregnancy and lasted for 9 days in two cases, and 4 days in another. In the only report of implantation bleeding in *M. fascicularis*, Macdonald (1971) cites implantation bleeding in five of ten pregnancies, beginning during the third week of pregnancy, and lasting for 10.8 days.

Ovulation Induction

The ovulation induction regime was initiated in an attempt to produce multiple follicles which then could be used to test the follicular puncture and aspiration system. The final injection of HCG was given at 9:00 P.M. on day 13 of the cycle, and examinations were begun at 6:00 A.M. the following morning. It was hoped that this schedule would provide sufficient time for pre-ovulatory follicles to develop, but which could be aspirated before ovulation occurred. As indicated in Table 9, this regime did produce a total of five characteristic pre-ovulatory follicles and nine cystic follicles; which, for the purposes of follicular aspiration, was considered successful. It was recommended, therefore, that the dosage regimen used in this trial could be employed in future attempts to aspirate pre-ovulatory follicles in *M. fascicularis*.

Follicular Aspiration

The major problem in the design of the follicular puncture and aspiration device was the development of a system which could be activated during laparoscopy by the laparoscopist alone. It was felt that such a system would not require the services of an assistant, as is currently in use by Steptoe and Edwards (1970). This would be of considerable advantage, since trials would often be conducted at inconvenient hours. It was also believed that such a system would be the most efficient since the person

who would be observing the procedure through the laparoscope would likely be the one who could time the puncture and aspiration most effectively. The system developed in the present study fulfills these objectives. Follicles have been incised and their contents aspirated. Follicular fluid has been collected (1.5 ml) and is being held for in vitro culture experiments.

The justification for this project is its critical importance to studies concerning the fertilizable life of the ovum, in vitro fertilization, capacitation, and a host of related investigations which would depend on the in vivo recovery of an ovum near the time when it would normally be ovulated. It now appears that further trials with the present system will provide the method to accomplish these objectives.

Temperature Variation in the Menstrual Cycle of *M. fascicularis*

As indicated in Figure 5 and Table 10, the range of temperature fluctuation when monitored by rectal thermometer varied from 0.7°C to 1.2°C. This extreme variability, and the erratic fluctuations throughout the cycle precluded the detection of a temperature shift which may be associated with ovulation in this species. The basis for this conclusion rests on the findings of cyclic temperature rhythms in the human female. In the human, ovulation is indicated by an elevation of 0.4°C above the base line (the average of the preceding 4 days), if followed by a persistent elevation

for a minimum of 3 consecutive days (Marshall, 1963). Such a marginal thermal shift would not have appeared in the recordings described in this experiment.

Behavior

A definite relationship between endogenous progesterone and estrogen levels and sexual behavior in the non-human primate has been established by several investigators (Michael et al., 1966; Erikson, 1967; and Vandenbergh, 1969). On this basis, a Reproduction Performance Index and Success Ratio were devised in order to provide an additional means of selecting females for breeding experiments, and as criteria for eliminating both males and females from the colony if required.

A screening process was carried out over a period of 6 weeks in order to identify and isolate various aspects of reproductive behavior. During this time, several means of quantifying the behavioral components were evaluated; and, at the end of 6 weeks, the present method of scoring reproductive performance was instituted for all mating trials.

As a means of evaluating the validity of this system, the R.P.I. and S.R. scores for each cycle length were plotted against the days of the cycle on which mating occurred, as in Figures 11, 12, and 13. If the system were functioning as anticipated, the R.P.I. and S.R. values for both males and females should exhibit trends related to the days of the cycle on which ovulation would be expected to

occur. Again, this pattern of reproductive behavior has been observed and confirmed by the investigators listed above.

Concerning the scores for 28 day cycles, there was a gradual decline of both Female R.P.I. and S.R. beginning on day 11 and progressing through day 14. This corresponds to what has been described by Michael et al. (1966); and to what one would expect if the female reproductive behavior is indeed related to endogenous estrogen levels which should be elevated during days 11 and 12, and declining through day 14. The corresponding Male R.P.I. and S.R. scores did not show a similar pattern on days 11 through 14, indicating that there was little variation in the measures used. The scoring system may not have been sensitive enough to emphasize the differences. The resolution of this question would depend upon the results of modifications of the scoring system which could then be compared with the present studies.

The female R.P.I. score for 30 day cycles showed a definite peak on day 14, which again suggests a correlation with female cyclicity. The S.R., however, showed a progressive increase from 10 to 30, which was not anticipated. Again, the male score showed little variation.

For the 32 day cycles, the female S.R. ratio exhibited a peak on day 16 which gradually declined on days 17 and 18, whereas the R.P.I. showed a progressive increase. The Male R.P.I. peaked on day 15, while the S.R. showed no change.

The results at this point indicate that the scoring system for female behavior is more sensitive to variations in sexual behavior related to cyclicity, than the scoring system for male behavior. The apparent contradictions in several cases between the peaks of the R.P.I. and S.R. scores, would perhaps be resolved by using females with carefully regulated cycles. It is suspected that the absence of clear and simultaneous peaks of R.P.I. and S.R. scores in 30 and 32 day cycles may be due to the accidental inclusion of other, probably longer, cycle lengths. As stated previously in the discussion of menstrual cycles, as cycle lengths become longer, an increasing percentage are found to be irregular. For this reason, it is believed that the present system, when related to cycle lengths of less than 30 days, illustrated a positive correlation to female cyclicity, and provided the best available criteria for evaluating females for breeding studies.

Laparoscopic Photography

The art of laparoscopic photography (Photo-laparography) involves a number of technical considerations. The most important piece of equipment is, of course, the camera. Three cameras were used during the course of the present studies. The Exacta had three distinct disadvantages. The field was inverted making manipulation of the reproductive organs a cumbersome procedure. Also, the viewer was located at the top of the camera so that, when

the laparoscope had to be raised during an observation, the investigator found himself at a disadvantage. Thirdly, the camera did not have a shutter speed between 1/8 and 1/30 second. On the positive side, the Exacta was capable of producing excellent results in spite of the difficulties of operation. Plate 3 is an example of a photograph taken with the Exacta. The Pen-F had a direct viewer and a 1/15 second shutter speed which were advantageous; but the double frame exposures (e.g., Plates 1 and 2) were sometimes inconvenient. The Canon TL had the advantage of the Pen-F without the double frame exposure. The viewer unfortunately had a focusing device which partially obscured the object to be photographed when one attempted to photograph at very short range. An example of photography with the Canon camera is seen in Plate 6, Figure 1. All cameras were, at various times, mounted on a tripod for additional stability, particularly if the shutter speed was slower than 1/15 second.

Ektachrome EHB-135 film was found to produce the most consistently satisfactory results. Other types of film often altered the natural color of tissues in a manner which made interpretation of the photographs difficult or impossible. The number 3 setting on the light projector was used for all standard color photography.

For infra-red photography, the following combination of filters was found to be the most effective: CC20-C, Corning CS - 1 59 (3966), and a Kodak Wratten #12. Ektachrome infra-red film was used for all trials, and the

number 2 setting on the projector was found to produce the proper light for the sensitivity of the film. Although the results demonstrated that infra-red technique offers several advantages over standard color film (Plate 4), the technique is not yet sufficiently developed to produce the maximum result. The maximum would be evidenced by a specific color rendition of biological tissue which has not yet been achieved. The present technique, however, has permitted the detection and clarification of minute vasculature associated with follicular development in *M. fascicularis*, and has pointed out the portion of the ovary which encompasses the early developing follicle (Plate 4).

Follicular Morphology

The science of laparoscopy obviates the need to perform complete laparotomies in order to examine the reproductive tracts in several species, including primates. Using serial laparoscopy in *M. fascicularis*, it was observed that ovarian follicles exhibited a very uniform and characteristic morphological pattern near the time of ovulation (Plates 1 and 2). The most notable component of follicular development, which provided the clearest indication of ovulation time and post-ovulatory changes, was found to be a vascular network which first became visible through the laparoscope approximately 30 hours before ovulation. This first appeared as a stellate pattern of vessels which resolved into a more specific arrangement as the follicular

cone emerged. Within 10 hours of ovulation, a single vessel became established across the center of the follicular wall. When the stigmata appeared on either side of this vessel, the follicle was considered to have reached its maximum pre-ovulatory development. Post-ovulatory follicular changes indicated in Plate 2 also permitted diagnosis of ovulation by laparoscopy.

The identification of the morphological development of the follicle in *M. fascicularis* has provided a valuable tool which can be used to identify early developing follicles, predict ovulation times, assist in the timing and recovery of ova by follicular aspiration, and evaluate the effects of pharmacological agents on ovarian function.

The physiological significance of the highly specific and unique vascular pattern is unknown. One can speculate that they may serve as a delivery system for collagenases or other enzymes which may play a role in the mechanism of ovulation; or perhaps the blocking of a specific vessel may result in a localized necrosis which might effect ovulation. The fact that such a distinctive anatomical pattern associated with follicular development has been identified should be of assistance to future investigators concerned with the mechanism of ovulation.

SUMMARY AND CONCLUSIONS

Studies were undertaken to characterize basic reproductive parameters, to establish a method of selecting females for breeding studies, to develop an in vivo system of follicular puncture and aspiration, to adapt photographic techniques to the laparoscope, and to characterize the follicular morphology in *M. fascicularis*.

The average menstrual cycle length was found to be 30.8 days with menses of 2.8 days. Of all cycles, 62.4% were regular with 45% of the animals having regular cycles at least 70% of the time. Gestation length was 164.5 days with implantation bleeding beginning on the 19th day of pregnancy, and lasting for 7.3 days.

The Female Reproduction Performance Index and Success Ratio were found to correlate with mating behavior related to cyclicity in females with cycles of less than 30 days.

Using a modified pediatric ear-suction, a follicular puncture and aspiration device was developed which was used to evacuate follicular contents and recover follicular fluid.

A photographic technique was established for laparoscopy. Both standard and infra-red film were used in the investigation of follicular morphology.

The anatomy of follicular maturation was observed and photographed through the laparoscope. It was found that morphological characteristics can be used to identify the site of the developing follicle, predict ovulation time, and diagnose ovulation.

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APPENDIX I

TAXONOMY NOTES: *MACACA FASCICULARIS*

APPENDIX I

TAXONOMY NOTES: *MACACA FASCICULARIS*

History of common names for *M. fascicularis* (Napier and Napier, 1967):

Crab-Eating Macaque	*Pithecus irus*
Cynomolgus Macaque	*Simia cynomolgus*
Javaneraffe	*Macacus carbonarius*
Java Monkey	*Macacus aureus*
Kra Monkey	*Macacus auratus*
Macaque de Büffon	*Innus cynomolgo*
Malayan Monkey	*Innus carbonarius*
Irus Monkey	*Inuus aureus*
Cerocebus cynomologo	*Macacus fur*
Pithecus fascicularis (mordax)	

Common accepted synonyms for *M. fascicularis*:

Cynomolgus macaque

Macaca irus

The term *Macaca fascicularis* was proposed for the crab-eating macaque by Raffles (1821) (cited in Miller, 1942).

APPENDIX II

REPRODUCTION PERFORMANCE INDEX AND SUCCESS RATIO SCORING SHEETS

APPENDIX II

DATA SHEET

Date: 9/2

Male: 28

Female: 41

DAY 13 of CYCLE

R. P. I. MALE (28)

Mounting attempts/trial — 6.0

Thrusts/mount (mean score) — 5.0

Vigor of thrusts (~~mean score~~) (Pelvic thrusts/sec(X) × 2) — 6.4

Presentations refused — - 2.0

RPI = 15.4

S. R. MALE

Male initiated mounts (6) / # Male mounting attempts (6) X 100 = 100%

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

R. P. I. Female (41)

Presentations — 3.0

Receptivity during mount (mean score) — 2.8

Refusals of male attempts to mount — -

RPI = 5.8

S. R. Female

Successful presentation (1) / Total # presentations (3) X 100 = 33.3%

Date: 9/2

Male: 28

Female: 5/1

THRUSTS/SEC: (2.4/sec)

MALE (28)

# Thrusts/mount:	1) 33 (14.0)	2) 16	(3.2) 3) 32	(4.0) 4) 19	(2.1) 5) 33	(2.4)
	6) 20	7)	8)	9)	10)
Duration of mount:	1) 12	2) 5	3) 8	4) 7	5) 14
	6) 5	7)	8)	9)	10)
Vigor of thrusts:	1) 3	2) 3	3) 5	4) 3	5) 5
	6) 5	7)	8)	9)	10)

Unsuccessful attempts to mount: _____

Presentations refused: 11

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

FEMALE (5/1)

Presentations (successful O): 11 — Successful presentations encircled

Refusals of male attempts to mount: _____

Receptivity during mount:	1) 5	2) 3	3) 3	4) 3	5) 5
	6) 3	7)	8)	9)	10)

Time of mating session: 1 15/min.

Comments:

APPENDIX III

PUBLICATIONS BY THE AUTHOR

APPENDIX III

PUBLICATIONS BY THE AUTHOR

Full Papers:

Laparoscopic Examination of the Ovaries in Goats and Primates, by W.R. Dukelow, S.J. Jarosz, D.A. Jewett, and R.M. Harrison. Laboratory Animal Science, 21: 594-597, 1971).

Follicular Morphology in *Maca Fascicularis*, by D.A. Jewett and W.R. Dukelow. Folia Primatologica 15:335-342, 1971.

Abstracts:

Ovulation and Cyclicity in *Macaca Fascicularis*.

Follicular Morphology and Ovulation Induction in the Non-Human Primate.

Laparoscopic Examination of Ovulation.

Follicular Morphology Near Ovulation in *Macaca Fascicularis*.

The Use of the Laparoscope and Precision Mating Techniques to Determine Gestation Length in a Non-Human Primate.

Brief Communications:

Laparoscopy and Precise Mating Techniques to Determine Gestation Length in *Macaca Fascicularis*, by D.A. Jewett and W.R. Dukelow. Laboratory Primate Newsletter 10:16-17, 1971.

See following pages for text of published papers.

APPENDIX IV

PUBLISHED PAPERS

LABORATORY ANIMAL SCIENCE
Vol. 21, No. 4

LAPAROSCOPIC EXAMINATION OF THE OVARIES IN GOATS AND PRIMATES[1,2,3,4]

W. RICHARD DUKELOW, S. J. JAROSZ,[5] D. A. JEWETT, AND R. M. HARRISON

SUMMARY • *A pediatric laparoscope was adapted for use in the African pygmy goat, squirrel monkey, and cynomolgus monkey. The technic allows the ovaries to be observed directly to determine time of ovulation and the effect of a contraceptive agent, and to assist in collecting samples directly from the ovary. Minimal restraint is required, and surgery is limited to small cutaneous incisions. With minimal prophylactic therapy there are no adverse effects even when the technic is used repeatedly on the same animal.*

In recent years the science of laparoscopy has achieved a high state of development (**1, 7**). This reflects the pioneering efforts of Semm (6) in the development of technics for ovarian biopsy, cyst removal, and sterilization (ligation of oviducts) with laparoscopy equipment. The present paper describes our experience with the adaptation of laparoscopy to reproductive physiology research in the African pygmy goat and several nonhuman primates.

MATERIALS AND METHODS

Laparoscope and ancillary equipment: The equipment used is illustrated in Fig 1. A Semm 135° pediatric laparoscope (Richard Wolf Co., Knittlingen, W. Germany) 5 mm in diameter was used. This instrument was attached to a Wolf Model 4000 Projector light source by means of a fiber optic cable. Insertion of the laparoscope was accomplished with a 6 mm trocar with the trocar sleeve fitted with a piston valve. Manipulation of internal organs was done with either a 5 mm tactile probe (goats) or a Verres cannula (primates). The laparoscope probes and trocar-cannula were kept in Zephiran-chloride® (Sterling Co., New York, N.Y.) 1:750 dilution prior to use since fiber optic systems should not be autoclaved.

Larger (8 and 10 mm) laparoscopes with varying fields of vision (135° and 180°) and

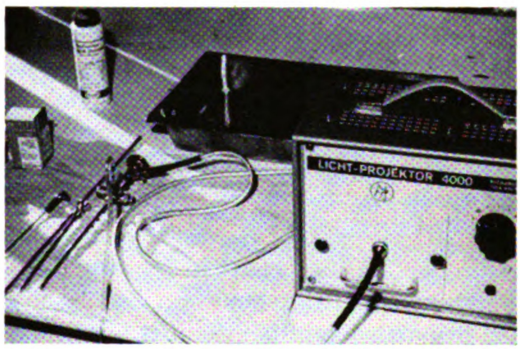


Fig 1. *Laparoscopy equipment (left to right): Verres cannula (small tactile probe), large tactile probe, trocar, telescope-cannula (with tygon CO_2 hose attached), fiber optic cable, and light projector.*

[1] From the Endocrine Research Unit, Center for Laboratory Animal Resources, Departments of Physiology and Animal Husbandry, Michigan State University, East Lansing, Michigan 48823.
[2] Presented at the 21st Annual Meeting, American Association of Laboratory Animal Science, Chicago, Illinois, November 6, 1970.
[3] Supported by NIH Contract No. 70-2061 from the Center for Population Research, USPHS Grant No. 5-PO6-RR00366-04 to the Center for Laboratory Animal Resources, and NIH Research Career Development Award No. 1-KO4-HD35306-01. Journal Article No. 5144, approved by the Director, Michigan Agricultural Experiment Station, Michigan State University.
[4] Accepted for publication March 1, 1971.
[5] Present address: W.S.R., Al. Mickiewicza 24/28, Krakow, Poland.

types of illumination (continuous light and electronic flash for photography) are available but were not utilized in the present experiments with relatively small animals.

Gas for abdominal insufflation was 5% CO_2 in either air or nitrogen, administered by way of the gas-connector valve on the trocar-sleeve.

Photography was accomplished by either: 1) an Exacta XV 1000, 35 mm single lens reflex camera with a 95 mm lens and laparoscope adapter, or 2) an Olympus Pen-F camera with the 95 mm lens and laparoscope adaptor.

Restraint of animals: The goats were tranquilized with promazine hydrochloride (Sparine®, Wyeth Labs., Philadelphia, Pa.) 1 mg/lb body wt im. Squirrel monkeys (*Saimiri sciureus*) were anesthetized with 15 mg pentabarbital sodium ip. Cynomolgus monkeys (*Macaca fascicularis*) were anesthetized with phencyclidine hydrochloride (Sernylan®, Parke, Davis & Co., Detroit, Mich.) 1.5 mg/lb body wt im.

Initially, attempts at goat laparoscopy were carried out with the goat in standing position in a stanchion; however, visualization and manipulation of both ovaries was found to be difficult with the pediatric type laparoscope used. Accordingly, the following procedure was adopted: A goat was placed on its back on a 45° sloped table (3) with the rear legs tied down, and a local anesthetic, 2% lidocaine with epinephrine, was injected subcutaneously at the sites of entry for the laparoscope and tactile probe. Primate species were restrained on a standard surgical table.

Laparoscopy: A small incision was made in the skin with a #11 scalpel blade at the mid-ventral point of entry in both goats and primates, and the trocar-cannula was inserted and passed posteriorally beneath the skin for 3–4 cm, then directed through the abdominal wall into the abdominal cavity. The trocar was withdrawn and the laparoscope inserted through the cannula. The fiber cable and the gas supply hose for abdominal distension were attached to the laparoscope and cannula, respectively (Fig 2).

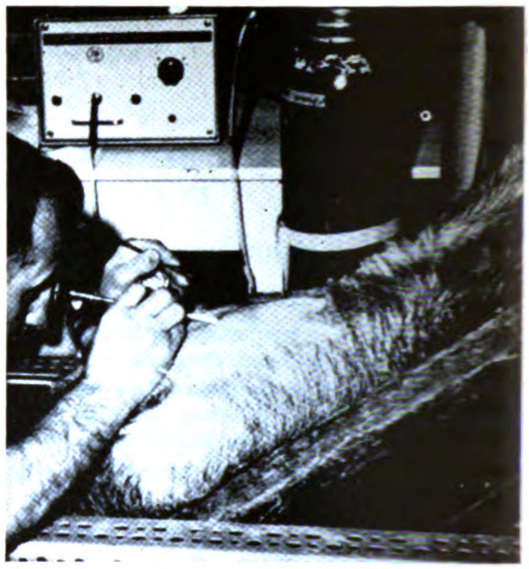


Fig 2. *Visualization of organs through telescope and manipulation of organs with large tactile probe in the pygmy goat.*

Upon completion of the examination, all instruments were removed and the puncture sites treated with Nitrofurazone® (Haver-Lockhart Labs., Kansas City, Mo.). In the smaller squirrel monkey, a single suture was placed in the skin wound. All animals received an im injection of 150,000 units of penicillin after examination.

To date, laparoscopy has been used literally hundreds of times in the species indicated with no adverse effects to the animal. In the goat, laparoscopy examinations were made as frequently as every 4 hr for several days through the same insertion point. If proper sanitary procedures are followed, no peritonitis or other infection occurs. Initially, some problems were encountered in the squirrel monkey with herniation through the laparoscope entrance incision; however, this was alleviated by the skin suture.

Frequent observation did not appear to affect the time of ovulation in the species studied (**2, 4, 5**), although confirmation of this will require more extensive studies. A

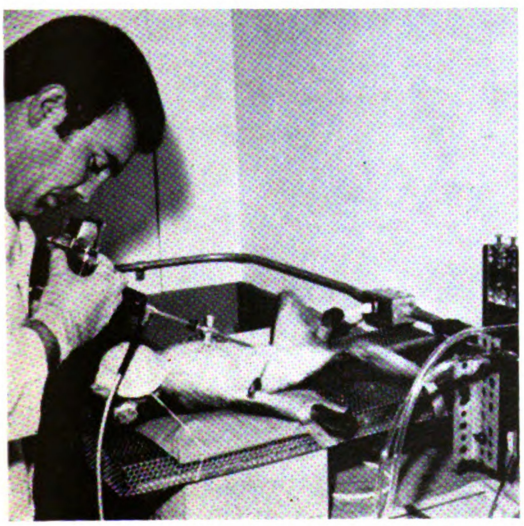
tactile probe was inserted with a similar procedure (6–7 cm in the goat, less in primates) to the left of the laparoscope.

Ovary visualization: Generally, 5% CO_2 in air or nitrogen was used to distend the abdominal cavity for better observation of the internal organs. Periodic insufflation was used, rather than continuous insufflation. The bladder was located and the tip of the laparoscope directed downward (dorsally) under the bladder. Because the ovaries of the goat are normally enclosed in the mesosalphinx and mesovarium, we found it necessary to lift the uterine horn with the tactile probe and look for the ovaries within these peritoneal folds.

By supporting the ovary by the ligamentum ovarium with the probe, one could observe the total surface of the ovary. In the primates, the ovaries are more easily located adjacent to the uterine fundus.

Photography: If photographs were desired, the camera was attached to the laparoscope (Fig 1). For photos taken in the cynomolgus, it was desirable to mount the camera on a tripod (Fig 3). Fig 4 illustrates the type of photography that can be accomplished and shows an ovary and developing follicle in the cynomolgus monkey 36 hr before ovulation.

Fig 3. *Photography through the laparoscope with Exacta camera on tripod. Verres probe shown to the right of the laparoscope entry point.*



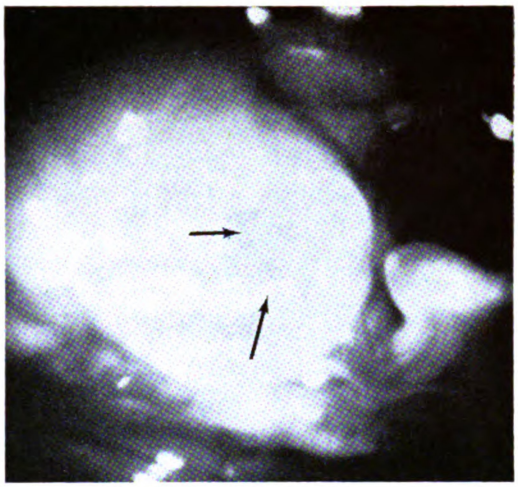


Fig 4. *Follicle on the ovary of the cynomolgus monkey 36 hr before ovulation, with oviductal fimbia to the left. Note the large coiled vessel on ovarian surface.*

Photography using the laparoscope is complicated by continually changing focus and illumination at the organ site, but with practice this handicap can be overcome. Our best results for projection slides have been achieved with Extachrome EHB film taken at a shutter speed of 1/15-⅛ sec with a camera stabilized on the tripod.

Processing the film at an ASA number of 400 (instead of the usual 160) did not significantly improve the quality of the slides.

The Ektacolor CPS-135 professional film shot at speeds of 1/15 and ⅛ sec appears to produce the best pictures for print production. Projection slides can be made from the negatives.

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Amer. Assoc. Lab. Animal Sci.
Chicago, Ill., Nov. 2-6, 1970

LAPAROSCOPIC EXAMINATION OF OVULATION[1]

W. Richard Dukelow, S. J. Jarosz,
D. A. Jewett, and R. M. Harrison[2]

Michigan State University

Determining the exact time of ovulation in domestic and laboratory animals has long been a problem in reproductive studies. Recently we have been using the laparoscope with good success to overcome this problem. Using this 135° pediatric laparoscope we have successfully examined the ovaries of rabbits, rats, pygmy goats, Toggenburg goats, guinea pigs, galagos, squirrel monkeys, and Java monkeys. Observations have been made as frequently as every 4 hr. for 32 hr. in pygmy goats without excessive irritation or peritonitis. Light tranquilization and a local anesthetic are required. A second entry tactile probe allows manipulation of the organs for observation. Distention of the abdominal cavity with CO_2/air is essential for good observation. Using this technique it has been possible to time ovulation within 1 hr. in over 60% of our monkeys and goats examined. The laparoscope offers an excellent tool for examination of internal physiological function with minor disturbance to the animal.

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[1]Presented at the 21st Annual Session, Amer. Assoc. for Lab. Animal Science Meeting, Chicago, Illinois, November 2-6, 1970.

[2]Endocrine Research Unit, Ctr. Lab. Animal Resources, Depts. An. Husb. & Physiol.

Fed. Proc. 30:2.
March/April 1971

OVULATION AND CYCLICITY IN *MACACA FASCICULARIS*[1]

D. A. Jewett, W. G. Elford, and W. R. Dukelow[2]
(Sponsor: W. G. Hoag, CLAR)
Michigan State University

Serial observations of follicular maturation by laparoscopy were made of pre- and post-ovulatory follicles in 31 female *M. fascicularis*. The laparoscope was used to characterize the normal cycle, estimate the lengths of the follicular and luteal phases, project the optimum time for recovery of follicular ova, and establish a time table for limited duration matings. The luteal phase is 13.6 days, whereas the more variable follicular phase is 15.0 days. Follicular development cannot be identified morphologically until 20 hrs pre-ovulation, when a generalized swelling of 35% of the ovarian surface appears. Vascularization of the follicular dome occurs 10 hrs pre-ovulation; partial luteinization, and formation of a stigma 6 hrs pre-ovulation, the formation of a corpus hemorrhagicum 6 hrs post-ovulation. Timing of ovulation has been used to facilitate the recovery of follicular ova for in vitro fertilization trials; and, in conjunction with single 30 min mating sessions, has resulted in 4 pregnancies.

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[1]Presented at the Fed. of Amer. Soc. for Exp. Biol. Meeting, New Orleans, La., March/April 1971.

[2]Endocrine Research Unit, Ctr. Lab. Animal Resources, Depts. An. Husb. & Physiol.

Society for The Study of Reproduction
Boston, Mass., June 30, 1971

FOLLICULAR MORPHOLOGY NEAR OVULATION IN *MACACA FASCICULARIS*[1]

D. A. Jewett, R. M. Harrison, M. P. Johsnon, and W. R. Dukelow[2]

Michigan State University

In the course of 189 laparoscopic examinations of 31 female Cynomologus Macaques *(Macaca fascicularis)*, ovarian morphology was observed and photographed for the purpose of determining the characteristics of pre- and post-ovulatory follicular development. Serial observations were begun on day 10 of the menstrual cycle and continued for 6 days at intervals varying from 6 to 24 hours. Generalized swelling and darkening of the ovary at the site of the developing follicle could not be identified until 20 to 24 hours prior to ovulation. The presence of a relatively large, coiled vessel near the follicular cone, smaller vessels around the base, and a single small vessel transecting the follicular membrane were found to be the most reliable indications of immediate pre-ovulatory development, and were not found earlier than 8 hours prior to ovulation. The most significant post-ovulatory morphological changes were the diffusion of the small vessels at the base of the follicular cone, the occlusion of clear areas in the follicular membrane, and

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[1]Presented at the Society for The Study of Reproduction Meeting, Boston, Mass., June 30, 1971.

[2]Endocrine Research Unit, Ctr. Lab. Animal Resources, Depts. An. Husb. & Physiol.

flattening or irregularity in the follicular dome. These changes were clearly evident 10 to 24 hours following ovulation; and, therefore, the corpus hemorrhagicum can be easily distinguished morphologically from the pre-ovulatory follicle. This investigation demonstrated that the time requirement for the formation of the follicular dome and related fasculature is not more than 20 hours; that ovulation can be diagnosed by laparoscopy; and, that comparative follicular morphology during the late stages of development will permit future accurate prediction of ovulation time by a single laparoscopic examination.

Canadian Assoc. Lab. Animal Sciences
Toronto, Canada, Nov. 10-12, 1971

THE USE OF LAPAROSCOPY AND PRECISION MATING TECHNIQUES TO DETERMINE GESTATION LENGTH IN A NONHUMAN PRIMATE[1]

W. Richard Dukelow and Dennis A. Jewett[2]

Michigan State University

Through the use of laparoscopy at close intervals beginning 18 to 20 hours before the expected time of ovulation, one can observe characteristic changes in the development of the follicle which enable approximation of the time of ovulation in *Macaca fascicularis*. Once the expected time of ovulation is known, females can be placed with fertile males for periods of time of only 20 to 30 minutes to allow mating to occur. Using these techniques we have successfully impregnated 5 adult females of our 31 animal colony. Three cases have been allowed to go to term and the gestation determined within a range of only a few hours. Sernylan anesthesia and laparoscopy did not appear to affect either implantation or the pregnancy when done on day 3 or 97 of a single animal. It is believed that this is the first report of the use of laparoscopy and short-time single mating sessions to precisely determine gestation length in a nonhuman primate and to use laparoscopy to diagnose pregnancy in this species.

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[1]To be presented at the Canadian Association for Lab. Animal Science Meeting, November 10-12, 1971.

[2]Endocrine Research Unit, Ctr. Lab. Animal Resources, Depts. An. Husb. & Physiology.

VIIth World Congress on Fertil. & Steril.
Tokyo and Kyoto, Japan, Oct. 17-25, 1971

FOLLICULAR MORPHOLOGY AND OVULATION INDUCTION IN THE NONHUMAN PRIMATE[1]

W. R. Dukelow, R. M. Harrison, and D. A. Jewett[2]

Michigan State University

Thirty-one female *Macaca fascicularis* were examined by laparoscopy to evaluate ovarian morphology and follicular development near ovulation. Generalized swelling and darkening of the ovary characterized the developing follicle 24 hr prior to ovulation; a specific pattern of blood vessels associated with the follicle developed 8 hr prior to ovulation. Deterioration of vasculature and occlusion of previously clear areas on the follicular membrane were evident 24 hrs after ovulation. Based on these examinations precise mating trials were carried out. Four pregnancies were obtained, and two were allowed to go to term with gestation lengths of 164 days, 15.5 hr, and 165 days, 10.8 hr.

Laparoscopy was also used to ascertain the efficacy of a *Saimiri sciureus* ovulation induction scheme consisting of 5 days of progesterone (5 mg); 4 days of FSH (1 mg); and, a final injection of 250 i.u. HCG. This scheme was used to study the effectiveness of various contraceptive agents in

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[1]To be presented at the VIIth World Congress on Fertility and Sterility Meeting, Tokyo and Kyoto, Japan, October 17-25, 1971.

[2]Endocrine Research Unit, Ctr. Lab. Animal Resources, Depts. An. Husb. & Physiol.

blocking induced ovulation. Such ovulation was completely blocked with 500 µg. megestrol acetate. The ovulation scheme was also effective in *Galago crassidaudatus*.

LABORATORY PRIMATE NEWSLETTER
10 (1971) 16-17

LAPAROSCOPY AND PRECISE MATING TECHNIQUES TO DETERMINE GESTATION LENGTH IN *MACACA FASCICULARIS*

D. A. Jewett and W. Richard Dukelow

Endocrine Research Unit
Michigan State University
East Lansing, Michigan

LAPAROSCOPY AND PRECISE MATING TECHNIQUES TO DETERMINE GESTATION LENGTH IN *MACACA FASCICULARIS**

D. A. Jewett and W. Richard Dukelow

Michigan State University

Using precise mating techniques and laparoscopy, two births of cynomolgus monkeys (*Macaca fascicularis*) have occurred at the Endocrine Research Unit at Michigan State University. The first mating (No. 54) occurred between 10:00 a.m. and 1:00 p.m., on July 31, 1970 (Day 14 of the cycle). Laparoscopy was not used immediately before or during pregnancy; however, such an examination was carried out under Sernylan (Parke-Davis) anesthesia at 11:00 a.m. on July 4, 1970. At that time one ovulation point, estimated to be 6 to 10 hours was observed. A normal female offspring was born between midnight and 6:00 a.m. on January 12, 1971. The gestation length was between 164 days, 11 hours and 164 days, 20 hours. Implantation bleeding was observed from August 18 to August 24, with moderately heavy flow.

The second female (No. 42) was mated between 10:55 a.m. and 11:25 a.m. on August 13, 1970 (Day 12 of the cycle). Laparoscopy was performed under Sernylan anesthesia on August 16, 1970 (Day 15 of the cycle) at 12:45 p.m. A newly formed corpus luteum was observed and photographed. On November 18, 1970 (Day 97 of pregnancy) at 10:00 a.m., pregnancy was confirmed by laparoscopic examination of the uterine fundus, circulatory vessels and ovarian ligaments. No adverse effects of either laparoscopy or Sernylan anesthesia on either Day 3 or Day 97 of pregnancy were observed. A normal female offspring was born at 9:15 p.m. on January 26, 1971. The gestation length was between 165 days, 10 hours, 30 minutes and 165 days, 11 hours.

Another female (No. 7) was subjected to precise mating and laparoscopy, but the pregnancy was terminated by cesarean section for other experimental purposes. Accordingly, the exact gestation length could not be determined. She was mated between 9:20 a.m. and 9:50 a.m. on September 15, 1970 (Day 11 of the cycle). Laparoscopy was performed at 9:30 a.m., September 16, 1970 (Day 12 of the cycle) and a fresh ovulation point was observed. Pregnancy was terminated on Day 92, December 16, 1970.

All three of the above pregnancies were by different males.

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Authors' address: Endocrine Research Unit, Laboratory of Comparative Reproductive Physiology, Center for Lab. Animals Resources, Michigan State University, East Lansing, Michigan 48823.

A fourth pregnancy was obtained (No. 16) with short-time mating, but the pregnancy was prematurely terminated. Unfortunately the laparoscopy records and early termination did not allow precise determination of ovulation time or gestation length.

Over 80 matings have been carried out with the four reported pregnancies. Matings have ranged from Day 11 to Day 15 of the cycle. This seemingly low level of fertility is due to the planned, wide variation in the time of mating relative to ovulation. These studies represent efforts to determine the fertilizable life of the cynomolgus monkey ovum *in vivo*, and will be reported in detail at a later date. This is believed to be the first report of the use of laparoscopy and short-time, single mating sessions to precisely determine gestation length, and of the use of laparoscopy to diagnose pregnancy in these animals.

* * *

* * *

VITA

Name:	Dennis Allen Jewett
Born:	November 25, 1943, Chippewa Falls, Wisc.
Formal Education:	Muskegon Community College Muskegon, Michigan, 1962-1965.
	Western Michigan University Kalamazoo, Michigan, 1965-67, 1969.
	University of South Carolina Columbia, South Carolina, 1968.
	Notre Dame University, South Bend, Indiana, 1969.
	Michigan State University East Lansing, Michigan, 1969-present.
Degrees Received:	Bachelor of Science Western Michigan University, 1970.
Experience:	Orthopedic Technician, Emergency Room at Bronson Medical Hospital, Kalamazoo, Mich. 1965-1967.
	EKG Technician, Intensive Care Unit, Columbia General Hospital Columbia, South Carolina. 1968-1969.