OVULATION IN MACACA FASCICULARIS

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ABSTRACT

OVULATION IN MACACA FASCICULARIS

By

Jon M.R. Rawson

Studies in Macaca fascicularis were undertaken to characterize the parameters of consecutive menstrual cycles from the same animal, to describe the relationship between the follicular phase and luteal phase of the menstrual cycle, to observe and photograph follicular development and ovulation, and to investigate the effects of clomiphene citrate and megestrol acetate on reproductive cyclicity and ovulation.

During the course of this study, 210 laparoscopic examinations were made during 115 menstrual cycles in 17 regularly cycling animals. The mean cycle length (±S.E.) was 30.8 ± 1.0 days with a median and a mode of 30 days. Comparing consecutive cycles from the same animal shows that 58.6% of the time, ovulation occurred on opposite ovaries while 42.4% had ovulation occurring on the same ovary during two consecutive cycles.

The relationships among the follicular phase, luteal phase and total cycle length were illustrated for 19 cycles where ovulation was determined within a 24 hour period. The mean follicular phase length was 14.0 ± 1.1 days with a range from 12.5 to 16 days and the mean luteal phase length was 15.6 ± 1.8 days with a range from 10.5 to 18.5 days.

The large number of laparoscopies performed during these studies had no effect on the cyclicity of the colony when compared to studies without laparoscopy. Laparoscopy was performed during pregnancy and lactation in one animal without interrupting either process. No follicular or luteal development was observed during pregnancy or lactation, and regular cyclicity resumed shortly after weaning.

The moment of follicular rupture was observed and photographed using laparoscopy, and the hypothesis that ovulation occurs at the stigma was confirmed. Typical pre-ovulatory follicular development consisted of either a blister-like stigma circumscribed by the follicular vessels or a large protuberant follicle having vascular elements raised from the ovarian surface. Abnormal morphology included a polystigmal follicle seen in 70% of the ovulations of one normal animal and twice in a clomiphene treated animal.

Administration of clomiphene citrate to induce ovulation in amenorrheic and regularly cycling macaques was quite successful with 75% ovulating within three cycles. Clomiphene-induced follicles were invariably larger than normal, and in several cases had abnormal appearances. A frequent consequence of this treatment was ovarian hypertrophy in the absence of ovulation.

Megestrol acetate did not block ovulation when administered to regularly cycling macaques, and follicular development was not altered.

OVULATION IN *MACACA FASCICULARIS*

By

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INTRODUCTION

The popularity of nonhuman primates as experimental models for anatomical, physiological, and pathological studies has greatly increased during the past several decades, a fact attributable to their close phylogenetic relationship with man. This affinity has been validated with respect to several body systems, but the close correlation among anatomical structures and neuroendocrine regulation of the female reproductive apparatus has indicated the particular value of this type of model. Following the realization that nonhuman primates were preferable to the classical laboratory animals for some types of experimental work, the delineation of their reproductive parameters was initiated. The greatest emphasis was placed on the rhesus monkey (Macaca mulatta) and even today relatively little is known about other nonhuman primate species.

Among those species that have not been widely studied are several which are now recognized to be preferable to the rhesus macaque for some studies. Such species include the squirrel monkey (Saimiri sciureus), the patas monkey (Erythrocebus patas), the chimpanzee (Pan troglodytes), the great apes (genus Pongo) and the cynomolgus macaque (Macaca fascicularis).

The cynomolgus macaque is a member of the family Cercopithecidae (Old World monkeys) whose geographical range includes the Philippine Islands, Indonesia and several Far Eastern countries. They have long,

non-prehensile tails and are generally smaller than the rhesus macaque with a weight range from 2.5 to 8.5 kg. Being Old World monkeys they exhibit menstruation which is not characterized by physiologic (summer) amenorrhea (Macdonald, 1971). They adapt well to laboratory environments and are becoming more extensively used due to their ease of handling and regular cyclicity.

Because of the similarity between the reproductive functions of M. fascicularis and the human, it seems that further delineation of the physiological functioning of this species would allow a better understanding of processes normally occurring during human reproductive cycle, as well as facilitating their laboratory breeding. The present studies used Macaca fascicularis as a model of reproductive function and were designed to characterize the parameters of consecutive menstrual cycles from the same animal, to describe the relationship between follicular phase and luteal phase of the menstrual cycle, to observe and photograph normal follicular development and ovulation, and to investigate the effects of clomiphene citrate and megestrol acetate on reproductive cyclicity and ovulation.

LITERATURE REVIEW

Due to the ever increasing bank of research data concerning primate reproduction, this review will cover primarily the animal used in the present study (Macaca fascicularis); a closely related and more widely studied species (Macaca mulatta); and selected references to the animal destined to benefit most from this research (Homo sapiens).

The first section of the review deals with accumulated menstrual cycle data and was assembled in tabular form to facilitate interspecific comparison. The remaining sections are sub-divided into the following categories: timing of ovulation; laparoscopy; normal ovulatory morphology; and drug effects on ovulation.

TABLE 1

Menstrual Cycle Data for Macaca mulatta (Mm) and Macaca fascicularis (Mf)

Study	Cycle Length[1]		Flow Duration[1]		Follicular Phase[1]		Method[2]
	Mm	Mf	Mm	Mf	Mm	Mf	
Heape 1897	29.5	31	3-5				
Allen 1927	10-70		4[3]				
Joachimovits 1928		25-29		3-5			
Spiegel 1930		30[3]					
Corner 1932		40.1		4[3]			
Hartman 1932	27.5		3.6		13		Isolated mating
Hartman 1933	25.3				13		Rectal palpation
Lewis & Hartman 1933					13.4		Rectal palpation

TABLE 1 (cont'd.)

Study	Cycle Length[1]		Flow Duration[1]		Follicular Phase[1]		Method[2]
	Mm	Mf	Mm	Mf	Mm	Mf	
Bryans 1951	26.2						
Spiegel 1954		28					
Erikson 1961	28.4±2.54						
Fujiwara & Imamachi 1966		23-37[4]					
van Wagenen 1966	29±3		4±1				
Fujiwara et al. 1967		28[3]		2			
Kerber & Reese 1969	35.4	47.2	2.6	2.6			
Fujiwara & Imamachi 1969		27.5[3]		2-3		12-17	Laparotomy

TABLE 1 (cont'd.)

Study	Cycle Length[1]		Flow Duration[1]		Follicular Phase[1]		Method[2]
	Mm	Mf	Mm	Mf	Mm	Mf	
Eckstein 1969	28.8						
Mahoney 1970		31±8.9[5]		3		14.1±3.1	Rectal palpation
Kaverne & Michael 1970	38.6±1.6						
Macdonald 1971	29.2±0.7	31.3±1.5	3.3±0.1	4.2±0.3			
Nawar & Hafez 1972		26-38		3-4			
Jewett & Dukelow 1972		30.8		2.8			

[1]Mean S.D.

[2]Method of detecting ovulation

[3]Mode

[4]Range of most cycles

[5]Calculated from text of study

Ovulation Timing

Except for delineation of menstrual cycle parameters (Table 1), the first major contributions to the study of ovulation in nonhuman primates were by Heape (1897) who suggested that menstruation in Macaca mulatta could occur without ovulation; and Van Herwerden (1906) who extended Heape's observations to Cercocebus cynomologo (Macaca fascicularis). Corner (1923) attempted without success to relate the time of ovulation to the menstrual cycle of Macaca mulatta using patterns of vaginal cytology.

Allen (1927) also attempted to diagnose cyclic patterns of vaginal cytology and, like his predecessor, concluded that Macaca mulatta showed cytologic changes that were less distinct than those found in rodents. Since that time, work on Macaca mulatta (Hartman, 1932; Erikson, 1968; Mauro et al., 1970) and Macaca fascicularis (Fujiwara & Imamachi, 1966; Mahoney, 1970; Nawar & Hafez, 1972) has substantiated the lack of correlation between primate vaginal cytology and the time of ovulation. In reviewing clinical data on the human female, Asdell (1927) stated that in 568 pregnancies the average period from an isolated coitus to parturition was 269.7 days, or 10.2 days less than the 279.9 day average interval from initiation of the pre-conception menstrual period to parturition. This 10.2-11.2 day interval (allowing one day for miscalculation of the first day of menstrual bleeding) was said to represent the time from initiation of menstruation to ovulation. Asdell also concluded that while the time of ovulation was variable, it occurred most frequently during the first two weeks of normal cycles. He further stated that the interval from menstruation to ovulation (follicular phase) was more variable than the interval from ovulation to the next menstruation (luteal phase).

In a discussion of contraceptive techniques, Dickenson (1927) reported that the highest level of human fertility occurs during the first 7 to 10 days after menstruation. Using ovarian observations during abdominal surgery he concluded that ovulation generally occurred between the fourteenth and the nineteenth days of the cycle.

Aside from these early attempts to discern the time of ovulation, there were relatively few techniques applicable to primates that gave reasonable results until Hartman (1932) reported 55 pregnancies in Macaca mulatta, each of which followed a single mating session. The majority of the sessions resulting in conception occurred between days 10 and 16 of the 28 day cycle with the average being on day 13. He also reported on the use of bimanual rectal palpation to estimate the ovulation time accurately without surgery. In concluding this study he stated that ovulation did not necessarily alternate with regularity between the ovaries.

In 1933, Hartman again reported on the technique of bimanual rectal palpation, this time with special reference to the timing of ovulation. In over 100 cases, ovulation occurred almost entirely between the tenth and the fifteenth days of the cycle. The data also suggested that any given female tended to ovulate repeatedly on the same cycle day. In contrast to the work of Asdell (1927) in humans, Hartman's data suggested that the follicular phase of the cycle interval is relatively constant, with variations in the total cycle length due largely to variations in the length of the luteal phase. Later in the same year, Lewis and Hartman (1933) recovered the fertilized ova from four animals whose mean ovulation time, diagnosed by rectal palpation, was day 13.4.

Since Hartman's first report of controlled matings, several other investigations have been undertaken to define ovulation time using this method. Van Wagenen (1945) reported on 160 pregnancies in Macaca mulatta having the highest fertility with matings between the eleventh and twelfth days.

Jacobson and Windle (1960) attempted to find the ideal mating time for M. mulatta and stated that there was a degree of variance in that time which was related to the total length of the menstrual cycles. Animals with cycles shorter than 26 days were said to have greatest fertility on day 9 of the menstrual cycle while day 11 was optimal for those with cycles between 26 and 28 days, and day 12 for animals having longer cycles.

Fujiwara and Imamachi (1966, 1969) unsuccessfully employed several diagnostic tests for ovulation (basal body temperature, vaginal cytology, cervical mucus properties, and rectal palpation), and concluded that a seven day mating session (day 11 to day 18) would best insure a satisfactory pregnancy rate in Macaca fascicularis.

Another investigation based on the isolated coitus method was reported by Valerio (1970) who corraborated Jacobson and Windle's theory that fertility varied with the cycle length. Attempting to obtain an optimum conception per mating ratio, it was found that in M. mulatta having 26 to 30 day menstrual cycles the most advantageous mating sessions were from days 11 to 14, animals with shorter cycles (24 to 25 days) should be mated earlier (day 10 to 13) and animals having longer cycles (31 to 33 days) should be mated later (day 12 to 15) in their cycles.

In a study designed to accurately determine the gestation length of the M. fascicularis, Dukelow et al. (1973) reported four pregnancies

obtained from short term mating sessions. The females were exposed to males for one half hour in three of the cases and for three hours in the fourth, with the mean conception date being day 13.

While controlled matings can be informative, they do not reveal the exact ovulation time due to several complicating factors (variation in ovulation time, gamete transport, fertilizable life of the gamete, etc.). Since the first attempts by Squire (1868) to correlate changes in basal temperature with human menstrual cycle events, there have been several significant studies which have established that a change in the temperature level is an indication that ovulation has occurred.

Van de Velde (1928) indicated that the higher temperature level seen in the later part of the menstrual cycle in women is due to the secretion of progesterone by the corpus luteum. Another corroborating report by Martin (1943) indicated that in women this increased temperature level coincided with the appearance of the secretory phase of the endometrium.

Attempting to define basal body temperature normality, Marshall (1963) analyzed records of 1,134 normal cycles in 155 healthy women. Statistically significant differences were found between the pre-ovulatory and post-ovulatory temperatures. Anovulatory cycles showed no differences in temperature levels. He also concluded that while the post-ovulatory phase varied, it was of relatively fixed duration compared to the pre-ovulatory phase.

Application of the basal body temperature recording method to M. mulatta and M. fascicularis was first attempted by Simpson and Galbraith (1906) who reported a diurnal variation. Erikson (1960)

successfully replicated this earlier study and additionally found that the temperature was increased by the stress of capture.

To circumvent any variation in temperature induced by external stress, Balin et al. (1965) developed a biotelemetry system capable of transmitting both deep body temperature and the temperature on the localized ovarian surface. Use of this system in M. mulatta (Balin and Wan, 1968) did not reveal accurately the moment of ovulation, even though a characteristic temperature trend was reported which was suggestive of ovulation.

Since ovulation is regulated hormonally, it is conceivable that an assay of the gonadotropic hormones could be used for a prediction of ovulation. The assay technique for these hormones is usually a double antibody method that requires a series of incubations and approximately six days for completion (Aono et al., 1967). Miyata et al. (1970) by decreasing the major incubation period from several days to two hours, sacrificed some sensitivity for a rapidity that had been previously impossible. Although they reported successful determination of the pre-ovulatory luteinizing hormone (LH) peak in the human, this assay has not yet been successfully adapted as a clinical aid. In 1970 Mahoney reported a study of the M. fascicularis menstrual cycle with special reference to the detection of ovulation. Employing rectal palpation, vaginal cytology, and cervical mucus chloride content techniques, he concluded that only rectal palpation could differentiate between ovulation and anovulation. The mean ovulation time for a group of 17 menstrual cycles was day 14.1 ± 3.1 with a luteal phase length of 17.3 ± 5.8.

The most reliable method of ovulation detection in the nonhuman primate is undoubtedly direct visual observation of the ovaries.

Without determining the average ovulation times, Johansson et al. (1968) and Betteridge et al. (1970) have discussed the morphology of the M. mulatta follicular apparatus near the time of ovulation. The latter report included photographs of the ovaries taken at laparotomy.

The application of fiber optic laparoscopy to this area by workers at the Endocrine Research Unit at Michigan State University (Dukelow et al., 1971; Jewett and Dukelow, 1971; Jewett and Dukelow, 1972; Dukelow et al., 1972), provided the foundation for the studies reported in this thesis. Without the trauma that necessarily accompanies a laparotomy, this technique was utilized to describe progressive maturation of the ovulatory follicle. The technique has also been used by Balin and Wan (1969) and Graham et al. (1973) to study the ovulatory processes in M. mulatta and Pan troglodytes, respectively.

Laparoscopy

The first reported attempt at visualization of an internal body structure was by Bozzini (1806) who employed a double-lumen urethral cannula to project candlelight.

Following the invention of the incandescent light bulb came the first successful illumination of an internal body by van Nitze (1878). By this time, endoscopic examination of the body cavities had become established even though the first clinical applications of this technique were not until Jacobaeus (1910) explored both the thorax and abdomen, referring to the procedures as thoracoscopy and laparoscopy, respectively. Nordentoeft (1912) made a significant contribution to the science of human laparoscopy when he utilized

gaseous distension of the abdomen and the Trendelenburg position to facilitate observation of the pelvic cavities. His descriptions of the female reproductive organs were the first to be obtained by this method and were of great practical importance.

During the next several years, the science of laparoscopy dealt mainly with the study and diagnosis of the pathological conditions of the internal organs. In 1952, Fourestier, Gladu and Vulmiere revolutionized endoscopy by developing a method of transmitting an intense light, by reflections from a powerful source along a quartz rod from the proximal to distal end of the telescope. This cool light removed the dangers of heat burning and internal dessication and allowed such a concentration of light as to permit not only black and white photography but also color photography and cinematography.

The first English laparoscopy textbook was written by Steptoe (1967) and a year later Fear (1968), outlined a number of clinical trials of the fiber optic type endoscope. Based on the principal that light was conducted along a transparent fiber cylinder due to its internal reflectance, this development added the feature of flexibility to the telescope.

It is to Semm (1969) that much of the credit for development and implementation of the laparoscope must be given. With his innovations, the technique of laparoscopy provided a means of direct visualization and photographic recording of the process of follicular development.

Using human subjects Steptoe and Edwards (1970) detailed the use of the laparoscope in the recovery of pre-ovulatory oocytes from the ovaries.

The laparoscope was first used to study ovulation in the nonhuman primate by Harrison and Dukelow (1970) who studied ovulation induction

in Saimiri sciureus and Galago senegalensis. The technique used by these researchers was fully described in 1971 (Dukelow, et al.) and then used to facilitate a study of the gestation length of M. fascicularis (Jewett and Dukelow, 1971). This laparoscopic technique has recently allowed photographic recording of the development of the pre-ovulatory follicle and of the post-ovulatory luteinization (Jewett and Dukelow, 1971, 1972; Rawson and Dukelow, 1972).

Normal Ovulatory Morphology

Commenting on the appearance of the pre-ovulatory follicles observed at laparotomy Johansson et al. (1968) stressed the variable appearance of the stigma which was observable within two days of ovulation. In 1970, Betteridge et al. took issue with these findings as they were unable to observe stigmal development within 24 hours of ovulation. They could not distinguish any changes in the mature follicles which would indicate when ovulation would occur.

Prior to aspiration of human pre-ovulatory follicles, Steptoe and Edwards (1970) described their appearance as thin-walled, bluish-pink, rounded swellings on the surface of the ovary, varying from 0.5 to 3.0 centimeters in diameter.

The first study designated solely to the study of follicular morphology near ovulation was published in 1971 by Jewett and Dukelow, utilizing serial laparoscopic observations. Their description of the normal ovulatory morphology included generalized swelling and darkening of the ovary at 24 hours before ovulation, development of a stellate pattern of blood vessels at eight hours, and development of a corpus hemorrhagicum following ovulation. In an extension of the same work in 1972, these authors described pictorally the development of the follicle before and after ovulation.

Drug Effects on Reproductive Cyclicity

Clomiphene citrate

The ability of clomiphene citrate (Clomid, W.S. Merrill, Cincinatti, Ohio) to induce ovulation in amenorrheic women (Greenblatt, 1961) prompted its use in nonhuman primates for two reasons. First, if effective in women it could be expected to be effective in other primates as an ovulation inducer. For this reason, Valerio and Courtney (1968) administered clomiphene to previously infertile M. mulatta (3 mg/kg for 3 days a month) and found that 56% of the treated animals became pregnant in less than three months. The second reason for administering clomiphene is to study its physiological activity as exemplified by Wan and Balin (1969, 1970). In these studies when compared to the common gonadotropin therapy, clomiphene (1.5 mg/kg 5 days a month) proved superior since ovarian hyperstimulation was avoided. Observing the ovarian morphology at laparotomy it was concluded that clomiphene and its isomers were more successful for inducing ovulation and in more closely simulating the normal ovulatory physiology.

Using clomiphene in Macaca fascicularis diagnosed anovulatory by rectal palpation, Mahoney (1970) found only five of fourteen cycles of treatment that resulted in ovulation when administered at a level of 3 to 5 mg/kg/day for five days.

Most of the research concerning the mechanism of action of clomiphene has been done in humans and despite its importance in modern clinical medicine, the physiological mechanism is still uncertain. One of its more interesting characteristics is its immediate anti-estrogenic effect on vaginal cytology and on the physical properties of cervical

mucus (Osmund-Clarke et al., 1968; Ruiz-Velasco et al., 1969). A rebound phase following the anti-estrogenic phase was described in women by Osmund-Clarke (1968), and several studies have shown that ovulation occurs during this period. Ruiz-Velasco et al. (1969) stated that ovulation followed cessation of clomiphene therapy (50 to 100 mg/day for 5 days) by approximately seven days. A corroborating report by Boutselis (1972) claimed that 75% of the 720 ovulations in the study occurred on days 7 or 8 following a 50 mg/day (5 days) regimen.

Keller et al. (1968) hypothesized a probable action on the hypothalamo-pituitary axis through modification or inhibition of the estrogen activity without significant influence on the ovary or the pituitary gland. Pennington's study (1969) of the inability of clomiphene to induce ovulation in women with high gonadotropin and low estrogen titers suggested the same conclusion as Keller; and Wan and Balin (1969) formed a similar result with M. mulatta.

Megestrol acetate

The first suggestion that low dosage progestational agents may be of importance to fertility control came from Martinez-Manautou et al. in 1966 when oral administration of 0.5 mg/day of chlormadinone acetate (17a-Acetoxy-6-chloro-6,7-dehydroprogesterone) was shown to inhibit fertility in women without supressing ovulation. This was attributed to a local antifertility effect due to a modification of the cervical mucus or the endometrium.

Using another progestational agent, megestrol acetate (17a-Acetoxy-6-methylpregna-4,6-diene-3,20 Dione), van Leusden (1969) reported the absence of cervical mucus ferning patterns and

spinnbarkeit following daily oral administration of 500 µg to women. Ovulation inhibition was not observed. Rudel (1970) found a significant increase in the antifertility potency of megestrol acetate when dissolved in an oil solution and injected into women.

The establishment of a reliable test system using the squirrel monkey Saimiri scuireus, by Harrison and Dukelow (1971) facilitated testing of the anti-ovulatory effects of doses of megestrol acetate. Their results show that administration of 500 µg daily for 5 days by subcutaneous injection in oil blocked ovulation completely, but doses of 250 µg or less did not.

MATERIALS AND METHODS

Animal Colony History

In January 1968 ten female and six male Macaca fascicularis were purchased from the Detroit Zoo. The colony was housed at the Veterinary Research Farm on the campus of Michigan State University, with 36 additional animals arriving in March of 1968. The purpose of this colony was to provide specimens for teratologic studies in the Department of Anatomy. It soon became evident that the maintenance cost of the animals exceeded their worth as originally intended, and the colony was moved in February 1969 to the Endocrine Research Unit where they could be maintained under controlled environmental conditions, and utilized in more dynamic research programs. The objectives of these programs were directed toward the physiology of reproduction, with special emphasis on the areas of ovulation and fertilization. The colony at this time housed 30 mature females, 6 mature males, and 7 infants.

Shortly after the transfer, it was decided to decrease the size of the colony. By selecting only those females that had exhibited regular menstrual cyclicity and those males that had proven their fertility, the colony was reduced to 21 mature females, one mature male, and three female infants at the start of the present research (August 1971).

Colony Care and Handling

The temperature in the colony was regulated at 21°C to 26°C with a cycle of 12 hours of light and 12 hours of darkness. The relative humidity fluctuated depending on the season (40 to 60%), the air flow was filtered and provided 12 complete air changes per hour.

The macaques were housed individually in 48-inch double unit modular cages. Each cage was equipped with a squeezeback apparatus to facilitate animal handling, a perch mounted on the back wall, and a false floor which allowed wastes to leave the cage rapidly and minimize contamination.

The cages were flushed daily, and sterilized at regular intervals. Each animal received water ad libitum from a bottle placed on the outside of the cage; they were fed once daily in the afternoon commercially prepared monkey diet (Wayne Co.). The animals were cared for by the staff of Center for Laboratory Animal Resources.

Menstrual Records

Visual inspection of the cages was made daily and the presence and relative amount of menstrual blood was recorded. The flow intensity was subjectively graded as light, medium or heavy on the basis of the amount of blood and on the physical state of the blood (color, degree of coagulation, etc.) in the cage. Day one of the menstrual cycle was considered to be the first day of bleeding. The regularity of the menstrual cycles was determined by the predictability of the onset of menses based on the mean cycle length of at least six previous menstrual cycles.

Laparoscopic Technique

Anesthesia was produced by an intramuscular injection of 15 mg (approx. 1.5 mg/kg of body weight) phencyclidine-HCl (Sernylan, Bio-Ceutic Laboratories). The animals were then placed in a deep Trendelenberg position, their abdomens shaved and washed with benzolkonium chloride (Zephiran, Winthrop), and a five mm incision made in the umbilical region. A trocar-cannula was inserted through the abdominal wall and the trocar removed. A 135-degree pediatric laparoscope (Richard Wolf Co., Knittlingen, W. Germany) was then inserted through the cannula and used to transilluminate the abdomen to insure proper placement of the Verres-cannula probe apparatus. Production of pneumoperitoneum using 5% CO_2 in air facilitated abdominal observation. The light source consisted of a Wolf model 4000 projector (Richard Wolf Co.) joined to the telescope by a fiber optic cable.

Photography through the laparoscope was accomplished with a Canon TL (35 mm single lens reflex) specially adapted to the telescope. Various shutter speeds (1/2 to 1/10 second) were used with Ektachrome EHB color film (ASA 120) to obtain highest quality photographs.

Following completion of the laparoscope examination, the instruments were withdrawn allowing the gas to escape and the incision site was closed with subcutaneous suturing. Nitrofurazone ointment was placed over the wound to prevent infection, and 150,000 units of penicillin were administered.

Normal Ovulatory Morphology

Prior to the administration of exogenous compounds it was necessary to characterize normal ovulatory morphology. To accomplish this,

laparoscopic examinations were begun between the ninth and twelfth days of the menstrual cycle, depending on the animal's normal cycle length, and continued until development of the corpus luteum was advanced (3 to 5 days). The same procedure allowed precise definition of the time of ovulation.

Surgical Recovery of Ova

On the suggestion of Dr. Richard Blandau of the University of Washington, it was decided that abdominal surgery would be performed in cases where the moment of follicular rupture was observed, in order to recover the ovum from the animal and check its physical relationship to the ovarian surface and the fimbria. Such surgery was performed during the course of these studies under Sernylan anesthesia.

Drug Administration

Clomiphene citrate

Clomid (W.S. Merrill Co., Cinncinati, Ohio) (Figure 1) was administered orally on an apple cube. The clomiphene (2 mg/kg/day) was given in the morning of five consecutive days, starting on an arbitrary day in amenorrheic animals and on day one of the cycle in normal animals.

In the case that ovulation and subsequent menstruation were not induced by the treatment, a new cycle was started 30 days following the first. Menstrual records were kept for these animals. The effect of the treatment on the ovaries was recorded by photolaparoscopy and compared to observations made during the cycle immediately prior to the drug administration.

Megestrol acetate

Megestrol acetate (17a-Acetoxy-6-methylpregna-4,6-diene-3,20 Dione; Mead Johnson) (Figure 2) was dissolved in oil and injected subcutaneously into animals at a dosage of 500 µg, a level reported to block ovulation in other primates (Harrison and Dukelow, 1971). Treatment was begun on either the eighth day of the cycle or the twelfth day and continued until day fifteen (eight and four day treatments respectively). The ovaries were examined before and after treatment by laparoscopy and important developments were photographed.

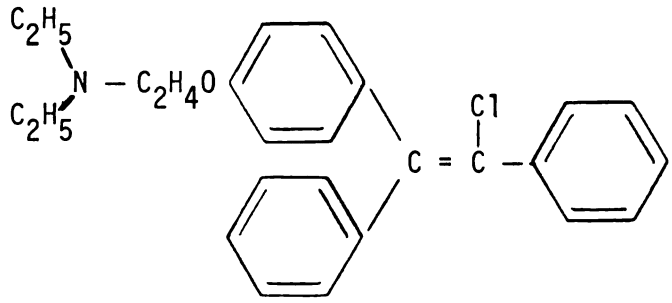


Figure 1. Clomiphene Citrate

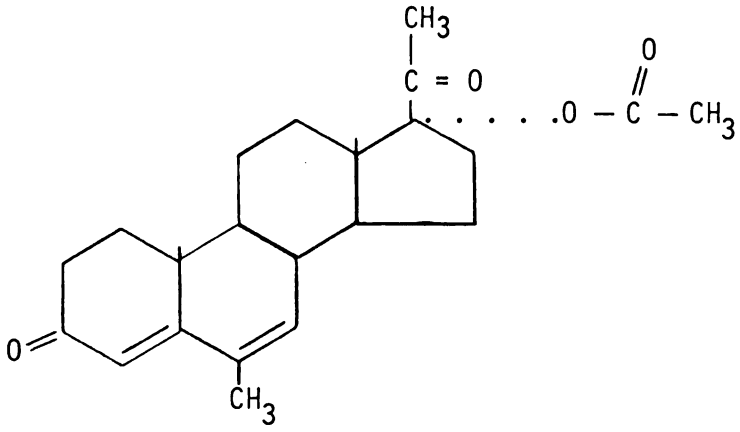


Figure 2. Megestrol Acetate

RESULTS

Reproductive Cyclicity and Ovulation Timing

During the course of this study, 210 laparoscopic examinations were made during 115 menstrual cycles in 17 regularly cycling animals (menses predictable within 4 days on the basis of past menstrual history). The mean cycle length (±S.E.) was 30.8 ± 1.0 days with a median and mode of 30 days.

Prior to this study, there had been no report describing ovulation in consecutive cycles from the same animal. While attempting to define the normal pre-ovulatory follicular morphology for individual animals, the data in Table 2 were collected. In 19 of 33 of these paired consecutive cycles (58.6%) the ovulation occurred on opposite ovaries while the remaining 14 (42.4%) had ovulation occurring on the same ovary in both cycles.

The relationships among the follicular phase, luteal phase and total cycle length are illustrated in Table 3, representing 19 cycles in which the time of ovulation could be determined within a 24 hour period. The mean follicular phase length was 14.0 ± 1.1 days with a range from 12.5 to 16. The mean luteal phase length was 15.6 ± 1.8 days with a 10.5 to 18.5 day range.

Another important determination that has not heretofore been discussed is the effect of the laparoscopy on the normal cyclicity of the animals. The fact that the menstrual cycles of these animals

TABLE 2

Ovulation Characteristics in Different Cycles Within the Same Animal

Female	Cycle Length[1]	Ovulation[2]	Side of Ovulation	Length of This Cycle	Luteal Phase
12	32*	15.5	L	33	17.5
	*	16	R	35	19
	*	13	L	33	20
	*		L	35	
18	*		R		
	*	15.5	R		
	*	13	R		
40	29.3	15	L	31	16
		12.5	R	29	16.5
	*	12.5	L	29	16.5
	*	14.5	L	30	15.5
	*	14.5	R	26	11.5
	*	15	R		
41	34*	14.5	L&R	34	19.5
	*	12.5	L	33	20.5
	*	14.5	L		
	†		R		
	†		R	36	
	†		L		
42	28*	12.5	L	29	16.5
	*	14.5	R	28	13.5
	*		L	32	
	*		R	28	
43	28	12.5	L	28	15.5
	*	14.5	L	25	10.5
	*	14.5	R	32	17.5
	*	14.5	R	30	15.5
	*	14.5	L		
44	29*	14.5	R	30	15.5
	*	13.5	L		
	*	14.5	L	29	14.5
	*	13.5	R	30	16.5
	*	15.5	R	31	15.5
	*	17.5	L	31	13.5
	*		R		

TABLE 2 (cont'd.)

Female	Cycle Length[1]	Ovulation[2]	Side of Ovulation	Length of This Cycle	Luteal Phase
53	31*	16	L	30	14
	*	13.5	R	30	16.5
	†	13.5	R	29	15.5
	†	14.5	L	31	16.5
	†	14.5	R	31	16.5
	*	13.5	L	32	18.5
	*	16.5	L	39	22.5
54	33*	15	R	31	16
	*	16.5	R	34	17.5
	†	15	R	31	16
	†	16.5	L	34	17.5
	†	14.5	L	34	19.5
	†		L		

[1]Mean of the six previous cycles

[2]Approximate day of ovulation when possible

*Denotes consecutive cycles

†Denotes a second series of consecutive cycles

TABLE 3

Relationship Between the Length of the Follicular Phase, Luteal Phase and Total Cycle Length of 19 Cycles.

Cycle Length (days)	Follicular Phase Length[1]	Luteal Phase Length[1]
25	14.5	10.5
26	12.5	13.5
28	12.5	15.5
28	12.5	15.5
29	13.5	15.5
29	12.5	16.5
29	14.5	14.5
29	14.5	14.5
29	13.5	15.5
30	16.0	14.0
30	14.5	15.5
30	12.5	17.5
30	14.5	15.5
30	14.5	15.5
31	15.0	16.0
32	14.5	17.5
32	13.5	18.5
32	15.5	16.5
34	15.5	18.5
	$\bar{X}^2$=14.0±1.1[3]	$\bar{X}^2$=15.6±1.8[3]

[1]When ovulation occurred between two examinations the follicular phase was considered as X ± 0.5.

[2]Not significantly different ($p < 0.10$)

[3]Standard Error

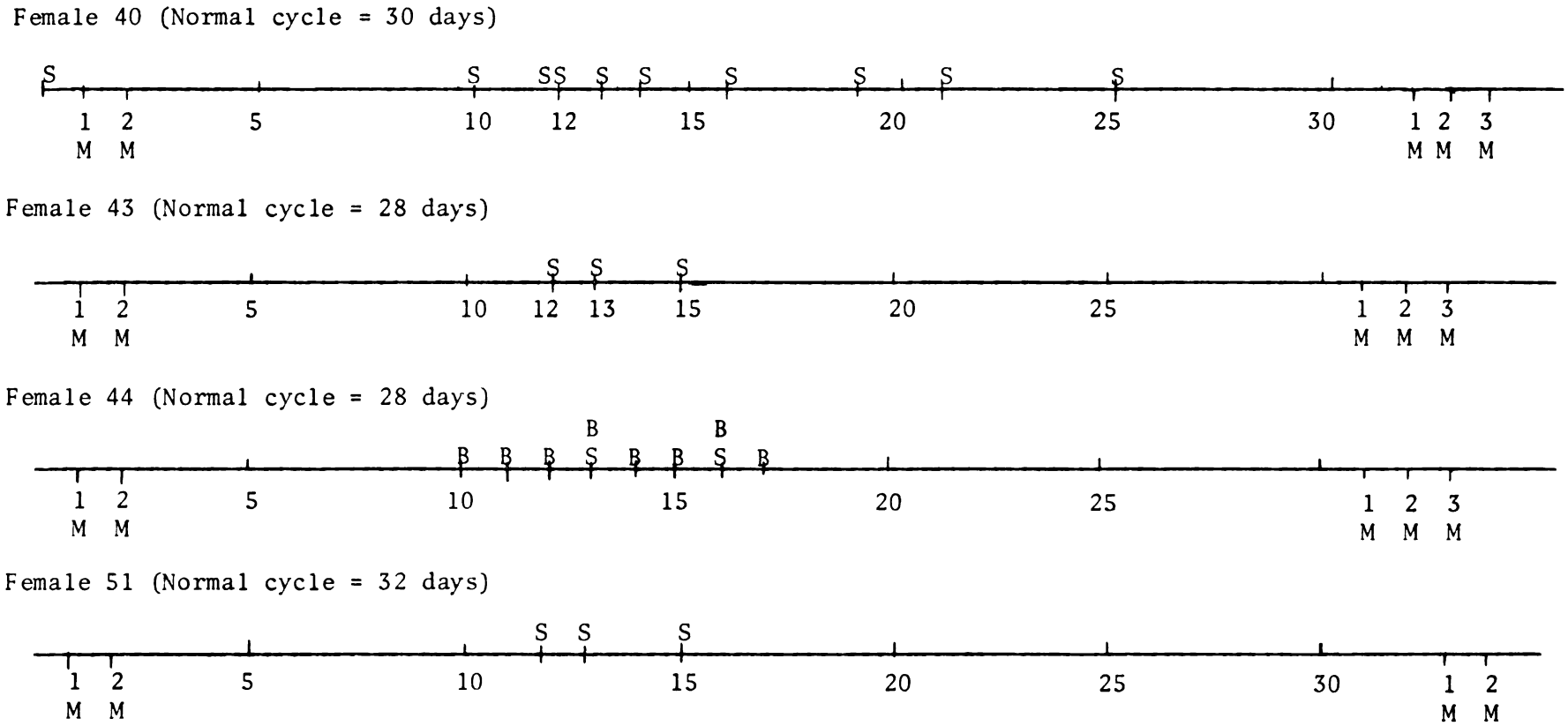
were of similar duration to those found in previous investigations (Table 1) suggests that the superimposition of a large number of laparoscopies on normally cycling animals does not alter their reproductive cyclicity. Furthermore, in several individual cases (females 40, 43, 44, 51; see Figure 3) the laparoscopic procedure did not alter the cycles of the animals. As a final example (Figure 4), one animal was anesthetized with Sernylan and blood samples were taken for 36 consecutive days. During this period, two laparoscopic examinations were performed. She ovulated and conceived on day 16 of her menstrual cycle (day 3 of anesthesia and venapuncture). Laparoscopy was performed during the seventh week of gestation and a gravid uterus was observed. On day 169 a normal female infant was delivered. Following parturition, the animal was laparoscoped six times (at 6, 10, 14, 16, and 22 weeks) without interrupting lactation, and the infant was weaned at 24 weeks of age. During lactation, menses were irregular with a gradually increasing interval between bleeding; eleven days following weaning menstruation began. The first post-conception ovulation was confirmed by laparoscopy on day 16 of the cycle and she also ovulated in the following cycle.

Normal Ovulatory Morphology

Although it is difficult to predict the exact structure of the ovulatory follicle at a given time, it is possible to describe the relative changes in follicular morphology before, during, and after ovulation. Follicles were often observed on the ovaries of the Macaca fascicularis as small, reddened, translucent spots. One of the first indications that a follicle would become the ovulatory follicle was the development of vascular elements on the ovarian surface in

FIGURE 3

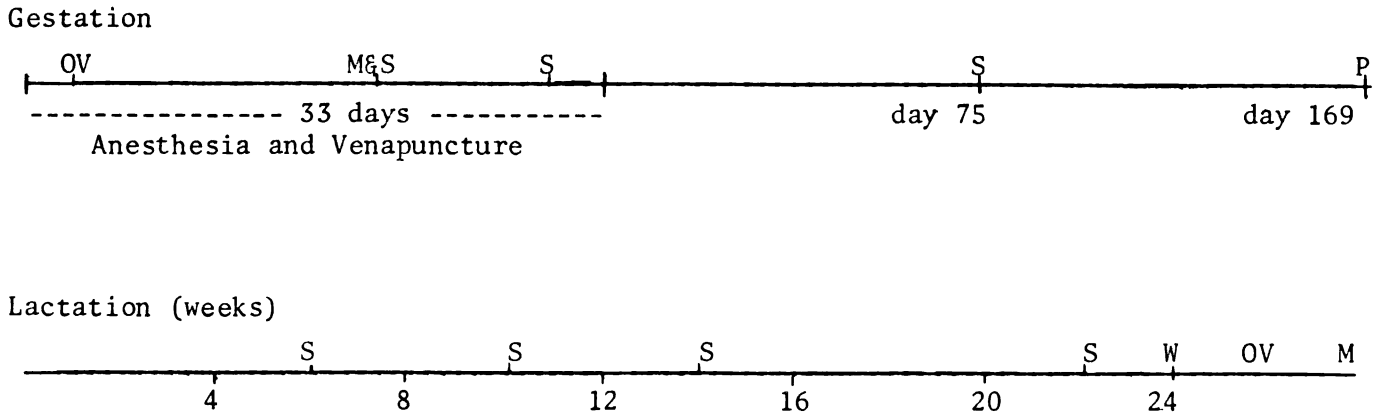
Effects of Serial Laparoscopy on Menstrual Cyclicity



S - Laparoscopic examination M - Menses B - Venapuncture

FIGURE 4

Gestation and Lactation of Female 52



OV - Ovulation

M - Menses

S - Laparoscopy

P - Parturition

W - Weaning

close association with the follicle. Ovarian enlargement and a darkening of the reddish color were other indications of follicular growth. These characteristics were usually observed 24 to 48 hours prior to ovulation.

Eight to twelve hours before ovulation, the vascular network was observed to develop to such a degree as to circumscribe a portion of the follicle from which the stigma will develop. The fully developed stigma appeared as a smooth, translucent, hemispherical structure.

The moment of follicular rupture (ovulation) was observed and photographed in three animals (Figures 5 and 6). These are believed to be the only photographs of observed ovulation in nonhuman primates, although others have observed rupture during laparotomy. Immediately prior to extrusion of the cumulus mass, the clear follicular fluid was observed protruding from the site of rupture (Figure 5).

Figure 5. Immediately prior to follicular rupture, follicular fluid (between arrows) can be seen protruding from the stigma.

Following expulsion of the cumulus mass the stigmal membrane collapsed and considerable bleeding from the follicle was observed. The entire cumulus mass could then be seen adhering to the ovarian surface (Figure 6). Although follicular rupture and release of the ovum was rapid, it was not the explosive phenomenon observed in the rat by Blandau, and it required approximately 30 seconds for completion. After laparotomy to recover the ovum, the viscid properties and adhesiveness of the cumulus mass allowed it to be stretched 1.5 cm before it could be freed from the ovarian surface. Under microscopic examination the ovum was recovered from the cumulus mass.

Figure 6. Immediately following expulsion of the follicular contents, the hemorrhagic cumulus mass (arrow) can be seen adhering to the ovarian surface.

During the course of these studies, two general types of pre-ovulatory follicular development were observed. In order to classify differences between individual follicles, they were labeled as type 1

or type 2 depending on their morphological appearance. Follicles were classified as type 1 if the stigma was large, clear, and protuberant (Figure 7). There were no vascular elements associated with the transluscent stigma. The follicular vessels are usually seen circumferential to the stigma conforming to the ovarian surface. Following ovulation the corpus luteum forms an extensive mass that does not greatly protrude from the ovary. Follicular boundaries are sometimes difficult to detect in a type 1 follicle.

The type 2 follicle generally encompasses a greater portion of the ovary than the type 1, and has a stigma that is smaller and less distinct than that of the type 1 follicle (Figure 8). The entire follicle is raised from the ovarian surface making the follicular boundaries quite distinct. Following ovulation, the corpus luteum develops into a highly protuberant yellow mass.

Table 4 illustrates ovulatory morphology of individual animals. Cases where classification was questionable have been omitted. From these 46 follicles 30 (65.2%) exhibited the pre-ovulatory morphological appearance consistent with a type 1 follicle, while 16 (34.8%) resembled the type 2 configuration.

It must be emphasized that these are generalized descriptions useful in standardizing classification. However, variations are observed in normal animals from time to time. One of the more interesting variations seen is illustrated by Figure 9. This poly-stigmal appearance has been observed to date in one normally cycling female. Five of seven follicles of this animal were of this type. In one additional, noncycling animal induced to ovulate with clomiphene citrate two of three follicles had this appearance.

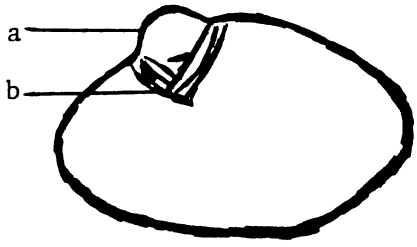


Figure 7. Type one follicle showing large, clear stigma (a) circumscribed by follicular vessels (b) (Photo - top view; sketch - lateral view).

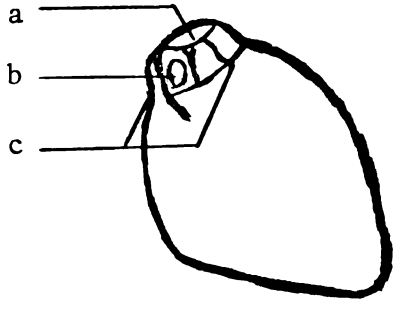


Figure 8. Type two follicle showing elevation of the follicular vessels (a) and the small clear stigma (b). The distinct follicular borders are also observed (c).

TABLE 4

Follicular Morphology

Female	Type 1	Type 2
11	*	
	*	
	*	
12		*
	*	
14	*	
	*	
	*	
17	*	
18	*(p)	
	*(p)	
40	*	
		*
	*	
	*	
	*	
41		*(L) *(R)
	*	
		*
	*	
42	*	
	*	
		*
43		*(L) *(R)
	*	
	*	
		*
44	*(p)	
		*(p)
		*
	*(p)	
	*(p)	
	*	
		*(p)

TABLE 4 (cont'd.)

Female	Type 1	Type 2
51	*	
	*	
	*	
		*
53		*
		*
	*	
		*
54	*	
	*	

p - polystigmal follicle

Figure 9. Polystigmal follicle observed in one normally cycling animal.

Drug Effects on Ovulation

Clomiphene citrate

Administration of clomiphene citrate to induce ovulation and menstruation in amenorrheic and in normally cycling animals was found to be quite successful. Figure 10 illustrates the time relationships between drug administration, ovulation and menstruation. Of the eight animals used in this study, six (75%) ovulated and menstruated within three treatment cycles. No clomiphene was administered during the rebound cycle.

In those animals that were previously amenorrheic, three of four responded to the drug treatment by ovulating during the first rebound cycle. The fourth animal required further clomiphene administration but did ovulate during the second rebound cycle. Laparoscopic examinations and uniformity of menstrual periods following ovulation give evidence that ovulation was induced at the normal time during the cycle.

Of the regularly cycling animals, only one ovulated during the first treatment cycle. Two of the remaining animals ovulated successfully during the rebound cycle while the fourth animals's cyclicity was interrupted by the clomiphene administration. These aniamls, like those in the earlier group, show uniform menses following the drug-induced ovulation. The possibility exists that the clomiphene was acting differently in these two groups of animals.

Laparoscopic examinations at intervals optimum for recording the drug effects on follicular morphology revealed that many small follicles were seen which did not ovulate and later seemed to disappear. Of those animals that did ovulate following clomiphene treatment, the follicles were invariably larger than normal, and in

FIGURE 10

Summary of Clomiphene Citrate Effects on Reproduction

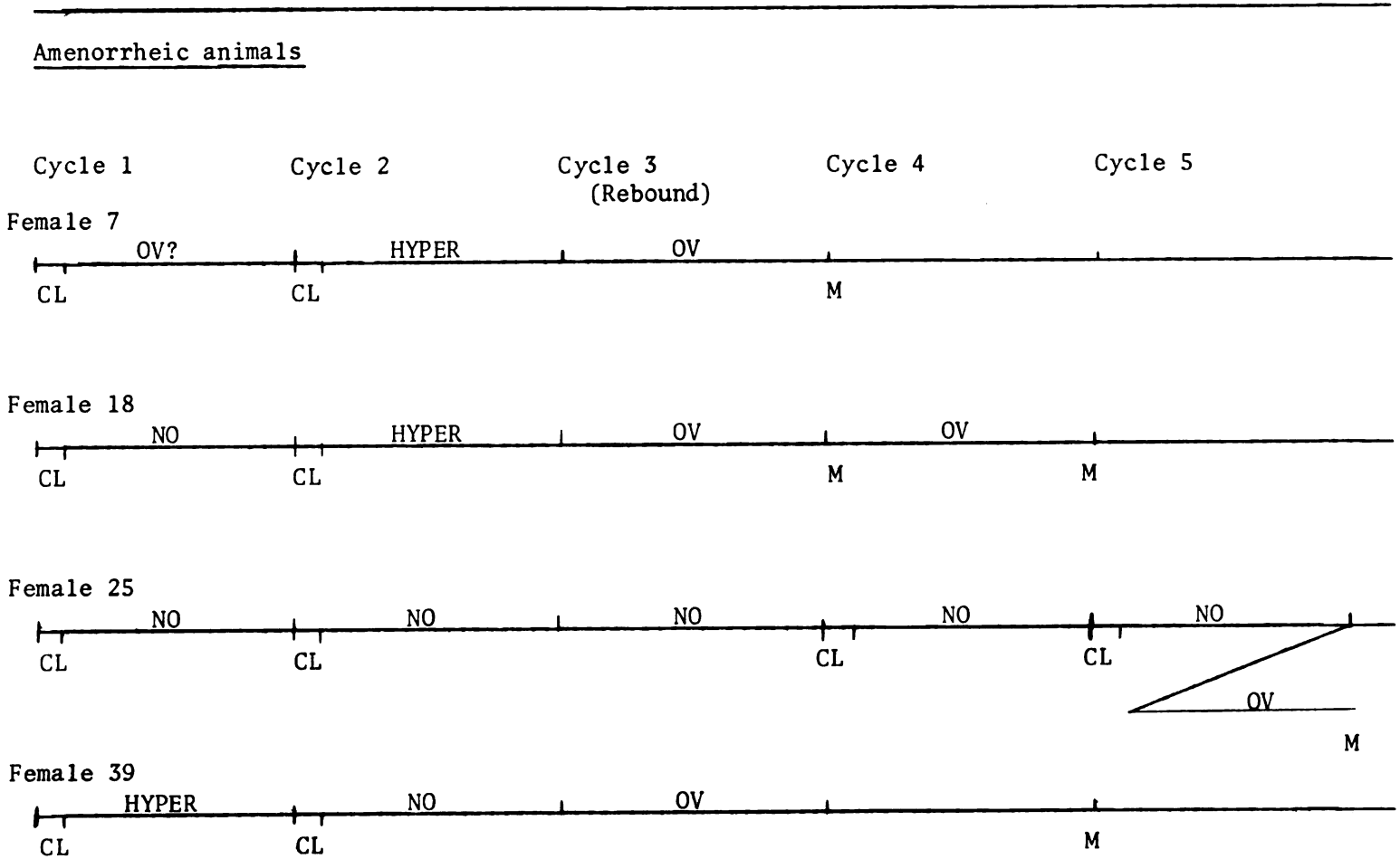
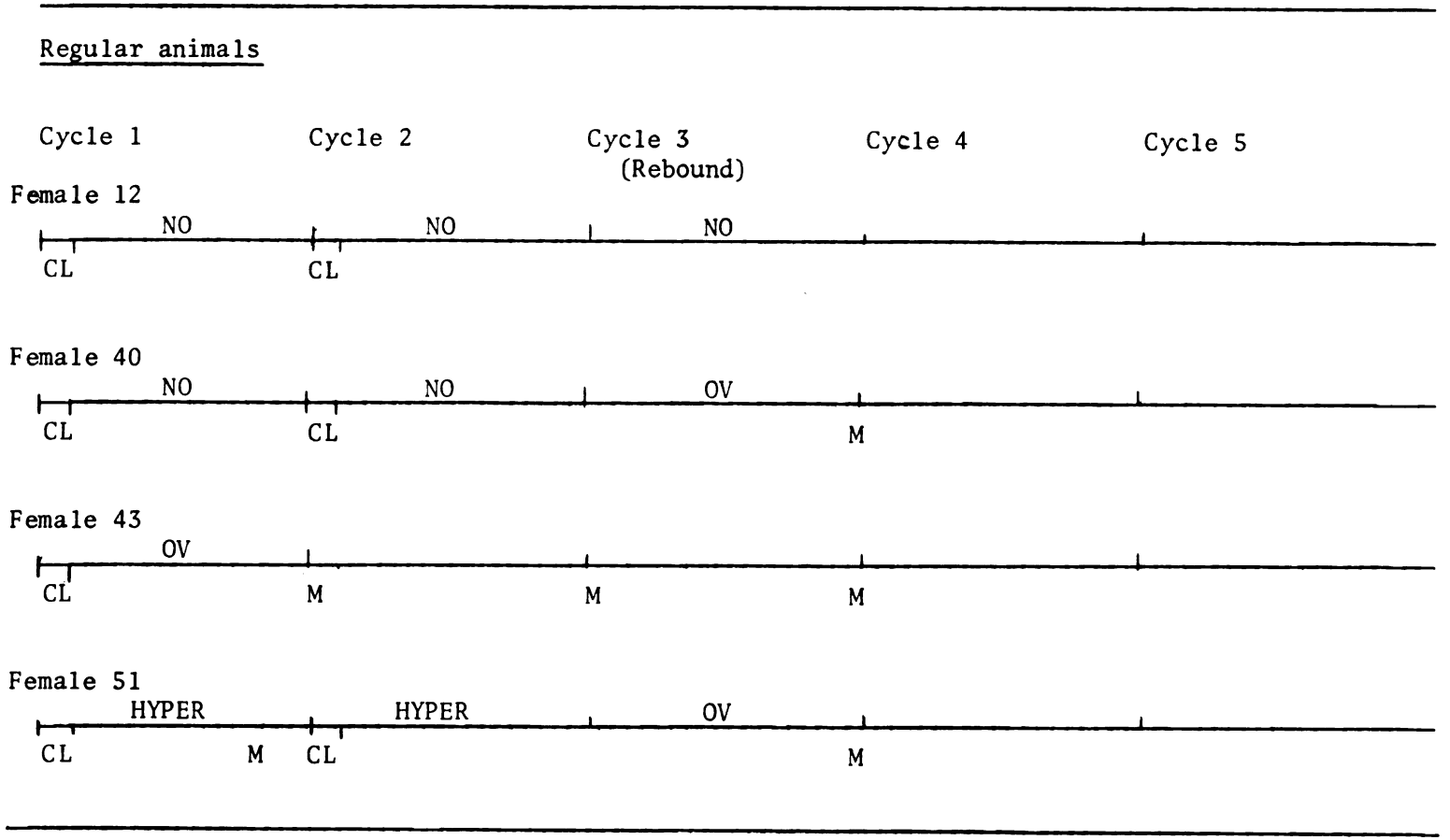


FIGURE 10 (cont'd.)



OV - Ovulation

HYPER - Hyperstimulation

CL - Clomiphene administration

M - Menses

several cases had abnormal appearances (Figures 9 and 11). Another frequent consequence of this treatment was ovarian hypertrophy in the absence of ovulation.

Figure 11. Abnormal ovulation following clomiphene citrate administration to an anovulatory macaque.

Megestrol acetate

The effects of megestrol acetate (MA) on follicular development and ovulation is another example of the type of study facilitated by the technique of laparoscopy. Of the three animals receiving MA for eight consecutive days two ovulated between days 14 and 18 of the treatment cycle (Figure 12) and again successfully in the subsequent, untreated cycle. The third animal did not ovulate until late in the cycle following MA administration. Of those animals receiving MA on four consecutive days, two ovulated during the treatment cycle, while the other two did not ovulate until the cycle following MA administration. Withdrawal bleeding occurred in all cases to verify the

FIGURE 12

Summary of Megestrol Acetate Effects on Reproduction

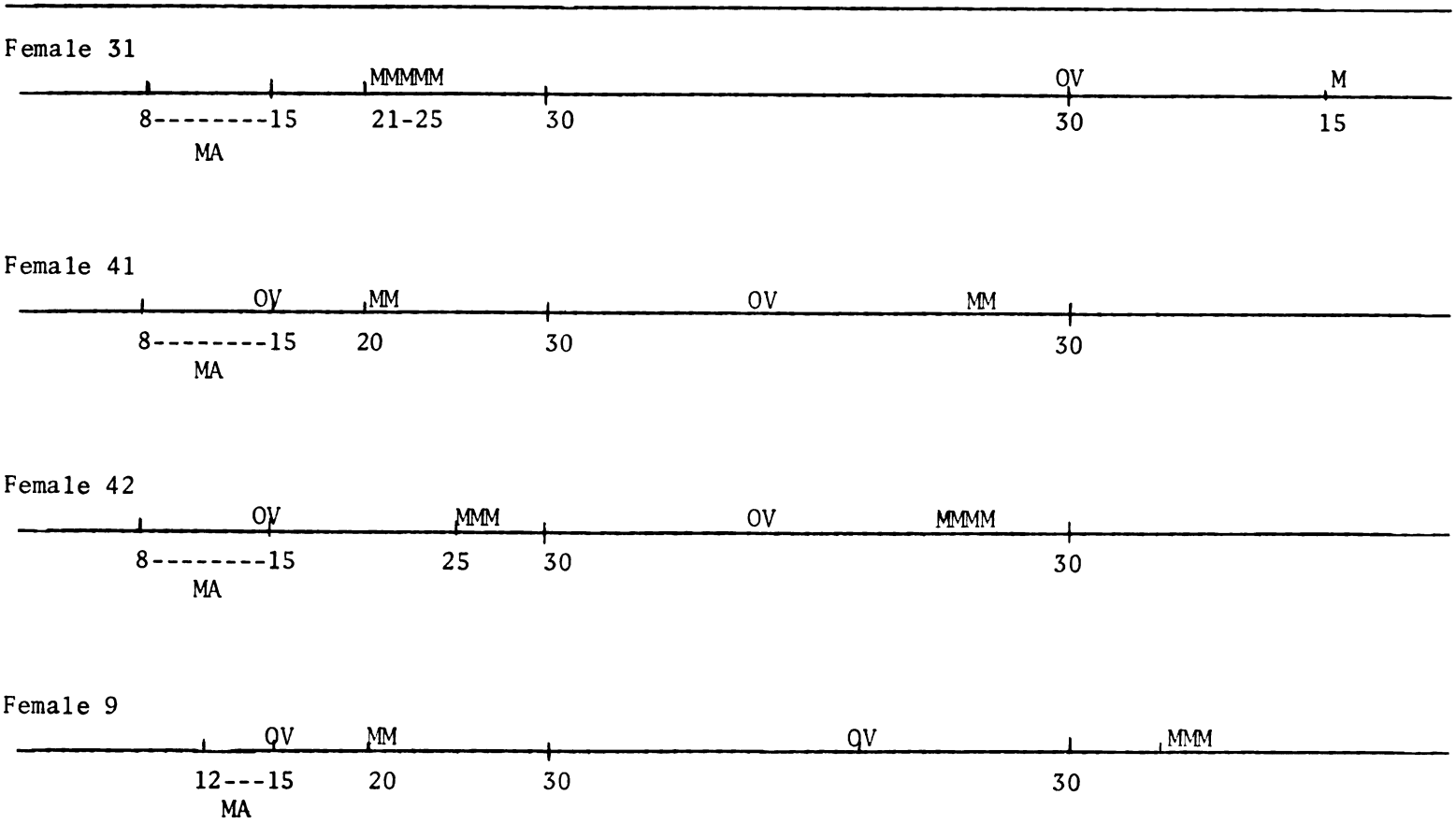
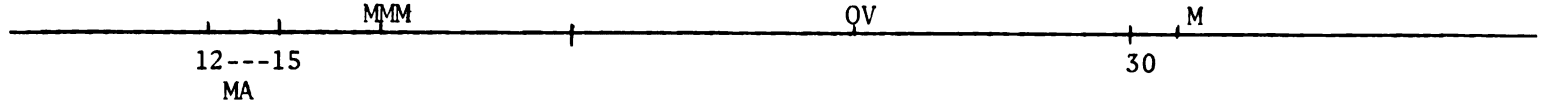
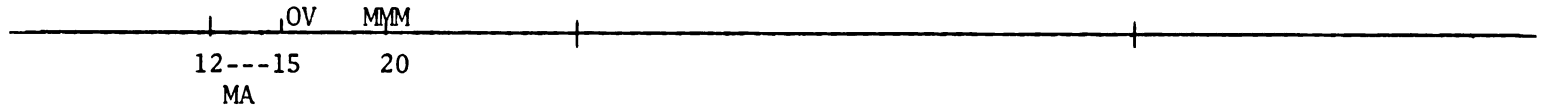


TABLE 12 (cont'd.)

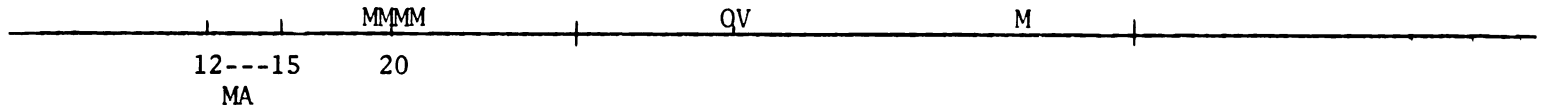
Female 14



Female 17



Female 53



M - Menses

OV - Ovulation

progestational action of the compound in the animal and normal cyclicity returned during the subsequent cycle.

The effects of MA on follicular development were minor, and included a possible decrease of follicular vascularity and delayed follicular rupture. All of those animals ovulating during the treatment did so between days 14 and 18.

DISCUSSION

Reproductive Cyclicity

The similarities among Macaca mulatta, Macaca fascicularis and the human female in regard to menstrual cycle parameters represents a major reason for the increasing usage of nonhuman primates in research.

The 30.8 ± 1.0 day mean cycle length of the macaques from the present study agrees closely with the averages previously determined (Jewett and Dukelow, 1972; Nawar and Hafez, 1972), as does the 30 day median and mode.

Calculations made from the data presented in Table 1 reveal that the average menstrual cycle length of the rhesus macaque (M. mulatta) is 27.9 days while the average of the crab-eating macaque (M. fascicularis) is 30.4 days. These calculations were made by omitting studies of each species in which the mean cycle data was inordinately high (Corner, 1932; Kerber and Reese, 1969; Kaverne and Michael, 1970). All of the studies deleted had mode cycle lengths that corresponded with the group modes (35.0 for M. f. from Corner, 1932; 28 for M. m. and 26 to 28 for M. f. from Kerber and Reese, 1969; and 28 for M. m. from Kaverne and Michael, 1970), and it was considered that a small number of amenorrheic or abnormal animals had skewed the average lengths. This agreement confirms the normality of the animals used while the data from Table 2 suggest that

ovarian alteration is not essential for normal cyclicity since a high percentage of the ovulations occurred on the same ovary during consecutive cycles. The fact that there is a similarity among the reproductive cyclicity of these three primate species suggests many questions concerning the feasibility of using the nonhuman primate as a model for the study of human reproduction. Differences between species do exist, such as the summer amenorrhea ("summer sterility") exhibited by Macaca mulatta (Riesen et al., 1971). Recently Macdonald (1971) concluded his study comparing three macaque species by stating that the M. fascicularis seemed to be an excellent model for study of the control of ovulation, implantation, pregnancy, parturition and the teratogenic effects of foreign agents because its reproductive function proved to be continuous and infrequently hampered by amenorrhea.

Ovulation Timing

Knowledge of the time of ovulation is very important when studying the reproductive physiology of an animal, and is necessary where accurate conception times or fertilizable gametes are sought. Investigations designed to detect the time of ovulation have shown that direct visualization of the ovaries offers the most accurate method of studying the temporal relationship between the follicular and luteal phases of the menstrual cycle. Presently there are only two methods of observing ovaries in vivo, laparotomy and laparoscopy. Studies which were designed to reveal the normal follicular structure at laparotomy (Johansson et al., 1967; Betteridge et al., 1970) were handicapped since only a few observations could be made during a cycle.

These workers did not discuss relationships between the follicular and luteal phase lengths.

Laparoscopy has provided a means of observing follicular development and predicting ovulation times without the trauma concomitant with abdominal surgery. In this study, ovulation timing was possible without altering the lengths of the menstrual cycles. The 14.0 ± 1.1 day follicular phase length observed confirms an earlier observation of a 14.1 ± 3.1 day follicular phase in the Macaca fascicularis revealed by rectal palpation (Mahoney, 1970). The luteal phase length in this study was 15.6 ± 1.8 days and did not differ statistically ($p < .10$) from the follicular phase length. From the data, it appears that ovulation in this animal occurs slightly before mid-cycle with a relatively stable follicular phase and a more variable luteal phase. Similar reports by Hartman (1932), who attributed almost all variation in cycle length of the M. mulatta to the post-ovulatory interval, and Mahoney (1970) who found a greater standard deviation in the luteal phase length than the follicular phase lengths of Macaca fascicularis, suggest that the growth and rupture of the follicle may require a shorter, more exact interval than does the formation, function, and lysis of the corpus luteum.

Since reproductive functioning can be interrupted by stress, as is often the case when performing laparotomies (Betteridge, 1972), it was necessary to evaluate the effects of the laparoscopic technique on cyclicity. The data in Table 5 compare the cycle parameters found during four separate investigations of Macaca fascicularis, using the first three as control studies for the present work. In none of the three control studies were the animals exposed to surgical procedures. The fact that 210 laparoscopic examinations during the

TABLE 5

Cycle Lengths and Ovulation Times of Cynomolgus Monkeys

Source	Mean Cycle Lengths	Ovulation Time	Technique[1]
Fujiwara et al. 1969	28.5±3.3 (S.D.) (N=272)	12-17 (N=4)	Laparotomy
Mahoney 1970	31.1±8.9 (S.D.) (N=25)	14.1±3.1 (N=18)	Palpation
Macdonald 1971	31.3±1.5 (S.E.)		
Present Studies	30.8±1.0 (S.E.) (N=115)	14.0±1.1 (N=19)	Laparoscopy

[1]Technique of Ovulation Timing

course of this study did not alter menstrual cyclicity suggests that the anesthesia and laparoscopic procedure are not a sufficiently stressful stimulus to interfere with normal reproductive functioning. In several of the animals where serial laparoscopic examinations were made during the same cycle, menstrual periods were normal. One animal that was anesthetized daily for a month and laparoscoped twice, ovulated, conceived, and delivered a normal infant after normal gestation. Further laparoscopy was not found to affect lactation.

Normal Ovulatory Morphology

Direct observation of the ovarian changes is the best method of determining whether or not ovulation has occurred. Hartman's studies using rectal palpation (1932, 1933, 1939) showed amazing accuracy when predicting the time of ovulation but were of little

value to the study of the physical nature of follicular development. Most previous attempts to observe the ovarian surface before and after ovulation, have been done at laparotomy, due to a lack of suitable technological means. One of these studies by Johansson et al. (1968) concluded that the appearance of pre-ovulatory follicles varies considerably but that the stigma could be found within two days of ovulation. Another study (Betteridge et al., 1970) took issue with this statement and, based on inability to observe stigmal morphology, suggested that if such changes occur in the rhesus macaque they must be abrupt.

Jewett and Dukelow (1971, 1973) using the same colony as the present study, adapted laparoscopy to the nonhuman primate and described the progressive development of the follicle before and after ovulation. These workers did not attempt to characterize the morphological structures observed during consecutive cycles in the same animals, and follicular rupture was not observed.

The present study confirms the descriptions of follicular enlargement prior to ovulation and the relative time sequence of events. The normal pre-ovulatory follicles can be observed in various stages of development for intervals of two to four days, and the specific arrangement of follicular vessels can often be observed for intervals of 15 to 24 hours.

The observation and photographic recording reported herein of ovulation in the nonhuman primate is the first report of its kind, although cinematography of follicular rupture has been recorded for both the rat and rabbit (Blandau, 1955). Photographs of ovulation and subsequent recovery of the ovum have confirmed the relationships

observed previously during this study and have shown that the point of follicular rupture is located on the stigma.

The fact that the cumulus mass is very adhesive and remains in close proximity to the ovarian surface immediately after ovulation suggests that the transfer of the ovum to the oviduct may occur as a result of the sweeping motion of the fimbria over the ovarian surface as observed in the rabbit. Another observation supporting this hypothesis is the close association of the fimbria to the pre-ovulatory follicles.

The predominance of the type 1 follicle during normal ovulatory cycles suggests that its characteristics may be those associated with normal ovulation. The other major type of pre-ovulatory follicular development occurs with sufficient frequency in normal cycles to suggest that its appearance is not abnormal. One can speculate that since these two types have been seen in the same animal, and even on the same ovary, the difference may be a function of the physical nature of the ovarian covering (the thickness or tenacity of the tunica albuginea), and the location of the developing follicle in the ovary (depth in the interstitium, or proximity to the suspensory ligaments).

Polystigmal follicles were observed in one female during 70% of her ovulations. Such abnormal morphology may reflect a variation in endocrine regulation. Further evidence that hormonal regulation may be the cause for this type of morphology comes from observations in an animal induced to ovulate with clomiphene citrate who responded with two polystigmal follicles out of three ovulations.

Drug Effects on Ovulation

Clomiphene citrate

The ability to induce normal ovulation in an abnormally cycling animal would be a valuable addition to the area of reproductive physiology. The induction of ovulation by clomiphene citrate was examined laparoscopically during this study. The results show the general applicability of laparoscopy to the screening of compounds of this kind. Ovulation was successfully induced in at least one cycle in 88% of the animals studied. All of the amenorrheic animals that were treated with clomiphene ovulated and, in all cases, the clomiphene induced single ovulations, which were characterized by large, in some cases abnormal appearing, follicles. The ovulations usually occurred mid-way in the third (rebound) cycle and were invariably followed by menstruation within 10 to 20 days.

These findings agree with those observed by Wan and Balin (1970) who found an ovulation rate of 94% in amenorrheic Macaca mulatta. Their hypothesis that the mechanism of action of clomiphene in these animals includes an anti-estrogenic inhibition of the hypothalamic negative feedback loop causing subsequent release of gonadotropins is supported by the present study.

Administration of clomiphene to regularly cycling animals resulted in a cessation of ovarian activity in 75% of the cases studied, a result inconsistent with the proposed mechanism of action. Wan and Balin attributed their failure to detect ovarian hyperstimulation to a possible species difference, or an effect of varying endogenous gonadotropin levels, a variation often seen in human patients (Kistner, 1965).

In view of the fact that ovarian hyperstimulation was observed during this study, and that cessation of ovulatory activity followed clomiphene administration in the previously normal animals, it is conceivable that their latter suggestion may explain the differences in response. If the anti-estrogenic effect of clomiphene in animals with a normal endogenous estrogen level is to inhibit the stimulation of gonadotropins at the pituitary level, such a cessation of activity might be expected. In any case, further study of the mechanism of action of this potentially valuable clinical aid are needed.

Megestrol acetate

Megestrol acetate (MA), an effective progestational agent, when administered at 500 μg daily on days 8 to 16 or 12 to 16 of the menstrual cycle was not effective in blocking ovulation in Macaca fascicularis. At this dosage, MA has been shown to be completely successful in blocking ovulation in the squirrel monkey (Saimiri sciureus) (Harrison and Dukelow, 1971). Daily administration of this level in women was shown to be an effective contraceptive program without blocking ovulation (van Leusden, 1969). The difference in response patterns among these species may reflect either the variation in animal size, since the squirrel monkey is approximately 10% as large as a mature macaque, or possibly a variation of endocronological regulation.

A recent study by Spies and Niswender (1972) indicated that when 500 μg of progesterone was injected into rhesus monkeys on days 2 to 10, 8 to 16 or 8 to 22, ovulation was inhibited in all cases. This apparent contradiction with present results may reflect a different biological activity between the progestational agents or a species difference.

SUMMARY AND CONCLUSIONS

The menstrual cycle parameters of Macaca fascicularis determined by laparoscopy during this study were in agreement with previous determinations employing other methods, and suggested that the large number of laparoscopic examinations did not alter their cyclicity. The 14.0 ± 1.1 day follicular phase length was not different from the 15.6 ± 1.8 day luteal phase length determined by laparoscopy but suggested that the luteal phase might be longer and more variable than the follicular phase. Alteration of ouvlation between ovaries was not indicated and in fact, data on paired cycles and on longer consecutive series suggested a random selection of follicular site.

Pre-ovulatory follicular morphology was photographed and categorized during consecutive menstrual cycles from individual animals. Two major types of development were described and follicular rupture was observed and photographed in three cases. Following observation of ovulation, laparotomy was performed and the cumulus mass was found to be closely adhered to the ovarian surface. The cumulus mass was removed from the ovarian surface and the ovum was found in its center confirming that ovulation had indeed just occurred.

Clomiphene citrate was successful in causing ovulation in at least one cycle in 88% of the animals studied. Clomiphene-induced ovulations usually occurred mid-way in the rebound cycle and were

followed by menstruation within 20 days. Ovarian hyperstimulation and abnormally appearing ovulations were frequently observed during treatment.

Administration of a progestational agent, megestrol acetate, during regular menstrual cycles did not inhibit ovulation or alter follicular development.

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APPENDIX A

PUBLICATIONS BY THE AUTHOR

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Full Papers: Natural and Artificial Control of Ovulation in Nonhuman Primates, by W.R. Dukelow, R.M. Harrison, J.M.R. Rawson, and M.P. Johnson. Medical Primatology, 1973. (Accepted for publication).

Follicular Development and Ovulation in Macaca fascicularis, Saimiri sciureus, and Galago senegalensis, by W.R. Dukelow, D.A. Jewett, and J.M.R. Rawson. American Journal of Physical Anthropology, 1973. (Accepted for publication).

Abstracts: The Environmental Enemy - Us, by J.M.R. Rawson. Presented at the First National Biological Congress, Detroit, Michigan, November 6-10, 1970.

Effect of Bone Marrow Depletion on Blood Volume of the Rat, by J.M.R. Rawson and J.R. Toepfer. Presented at the American Institute of Biological Sciences Regional Meeting, Erie, Penn., 1971.

Ovulation Morphology in Nonhuman Primates, by J.M.R. Rawson and W.R. Dukelow. Presented at the Michigan Academy of Science, Arts and Letters, East Lansing, Mich., March 23-25, 1972.

Comparative Ovulation in Nonhuman Primates, by W.R. Dukelow and J.M.R. Rawson. Presented at the Federation of American Societies for Experimental Biology Meeting, Atlantic City, N.J., April 1972.

Natural and Artificial Control of Ovulation in Nonhuman Primates, by W.R. Dukelow, R.M. Harrison, J.M.R. Rawson, and M.P. Johnson. Presented at the Third Conference on Experimental Medicine and Surgery in Primates, Lyon, France, June 21-23, 1972.

Follicular Development and Ovulation in Macaca fascicularis, Saimiri sciureus, and Galago senegalensis, by W.R. Dukelow, D.A. Jewett, and J.M.R. Rawson. Presented at the Fourth International Congress of Primatology, Portland, Oregon, August 15-18, 1972.

APPENDIX B

ABSTRACTS

COMPARATIVE OVULATION IN NONHUMAN PRIMATES[1]

W.R. Dukelow and J.M.R. Rawson[2]
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Previous studies in our laboratory have demonstrated the use of photolaparography to depict finite changes in follicular morphology during the reproductive cycle of Macaca fascicularis. In recent studies we have attempted to depict the comparative morphology of follicular growth to the time of ovulation and through corpus luteum formation in Saimiri sciureus and Galago senegalensis as well as M. fascicularis. Vascular patterns appear on the follicular surface in all three species within 30 hr of ovulation. The vessels cause a distinct division of the follicle into hemispheres in approximately 80% of the cases with the subsequent development of two translucent areas of the follicle, one of which will represent the point of rupture (stigma). After ovulation the corpus hemorrhagicum is of variable morphology ranging from a discrete single ovulation point to the classical, blood-filled, collapsed follicle seen in rodents. In M. fascicularis the majority of ovulations occur without rupture of the follicular vessels and these persist throughout the establishment of CL. In S. sciureus the CH is more hemorrhagic in appearance than M. fascicularis.

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[1]Presented at the Fed. of Amer. Soc. for Exp. Biol. Meeting, Atlantic City, N.J., April 1972.

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NATURAL AND ARTIFICIAL CONTROL OF OVULATION IN NONHUMAN PRIMATES[1]

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Ovulation occurs in response to endocrine changes induced by the reproductive cycle, the environment, or both. The present studies outline the progressive development of the follicle in Macaca fascicularis as the moment of ovulation approaches and characterizes morphological variation observed in the follicular vascularity and the formation of the corpora hemorrhagicum. Comparative follicular morphology has been studied by photolaparographic techniques in M. fascicularis, Saimiri sciureus, and Galago senegalensis. Using laparoscopic techniques to predict the time of ovulation, and precise-mating techniques for a limited period of time, pregnancies have been obtained.

Because of the increased use of S. sciureus in biomedical research and the strong contrast between native and captive environments (equatorial and temperate) we have studied the effectiveness of an induced ovulation scheme on animals in captivity from 2 weeks to 2 years, as well as the effectiveness of a progestational compound on blocking ovulatory response.

Ovulation in M. fascicularis is characterized by distinct changes occurring 24-36 hr before ovulation. These involve bifurcation of the

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[1]Presented at the Third Conference on Experimental Medicine and Surgery in Primates, Lyon, France, June 21-23, 1972.

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follicle by follicular blood vessels and (8 to 10 hr before ovulation) the appearance of stigmata. At the same time the fimbriated end of the oviduct positions itself adjacent to the developing follicle to facilitate ovum pick-up. Vascular patterns vary from small diffuse veins (S. sciureus) to distinct vessels dividing the follicles into 2 hemispheres (M. fascicularis). The appearance of the corpora hemorrhagica can vary from precise ovulation points and blood-filled follicles to a discrete "patchy" appearance on the follicular wall. Vessel patterns persist through corpora luteum formation. Using the laparoscope and precise-mating techniques, 5 pregnancies have been obtained in M. fascicularis, which occurred between Days 11 and 15 of the cycle (or, on the basis of the luteal phase, 16 to 18 days before the next expected menses).

The effectiveness of our ovulation regime in S. sciureus (J. Reprod. Fertil., 22:303, 1970), was found to vary by season of the year with a minimal number of animals ovulating in the summer months. At other times of the year, a good ovulatory response was obtained but could be effectively blocked with injections of 500 µg megestrol acetate (MA) daily. Lower doses inhibited ovulation to a lesser degree. MA could also be administered by subcutaneous silastic implant with a rate of release of approximately 50 µg/day.

These studies have bearing on the reproductive physiology of captive nonhuman primates especially relative to their use in the testing of contraceptive compounds.

FOLLICULAR DEVELOPMENT AND OVULATION IN MACACA FASCICULARIS, SAIMIRI SCIUREUS AND GALAGO SENEGALENSIS[1]

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During the 24 to 36 hrs before ovulation discrete changes occur in a fixed sequence which allow the prediction of ovulation in macaques. These changes can be observed using photolaparographic techniques we have described earlier (Lab. Animal Sci. 21:594, 1971; Folia Primat. 16:216, 1971) and involve bifurcation of the follicle by blood vessels and (8 to 10 hrs before ovulation) the appearance of stigmata. The fimbriated end of the oviduct positions itself adjacent to the follicle to facilitate ovum recovery. Vascular patterns on the follicular surface vary from stellate, complex patterns to single vessles and from small diffuse veins (S. sciureus) to pronounced vessles (M. fascicularis). Vascular patterns are also seen in G. senegalensis but are less pronounced than in M. fascicularis.

The appearance of the corpora hemorrhagica can vary from precise ovulation points and blood-filled follicles to a discrete "patchy" appearance on the follicular wall. Vessel patterns can persist through corpus luteum formation and has been observed on 8 day old corpora lutea.

Using laparoscopic prediction of ovulation time one can plan precise matings. Using 20 to 30 minute periods of exposure to the male (M. fascicularis) we have obtained five pregnancies in our colony of 22 females. The pregnancies occurred between days 11 and 15 of the cycle (or, 16 to 18 days before the next expected menses).

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[1]Presented at the Fourth International Congress of Primatology, Portland, Oregon, August 15-18, 1972.

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