

4. Title: Gibbon Menstrual Cycle and Breeding

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OBJECTIVE

The objective of this study is to study the menstrual cycle of the female gibbon, the semen of the male gibbon, and to obtain reproduction of the gibbon in the laboratory environment.

DESCRIPTION

The female gibbon is being studied to characterize the rhythm of the menstrual cycle and to associate periodic changes in the genitalia with the occurrence of ovulation. Present methods of study being employed are direct observations of both internal and external genitalia, vaginal swabs to detect menstrual bleeding, Papanicolaou stains of vaginal smears, histological examination of endometrial biopsies, and the regulatory effects of certain drugs. The electroejaculator is used for collecting gibbon semen samples and the semen is evaluated by standard methods for volume, pH, concentration, motility, viability, and density of spermatozoa.

PROGRESS

Vaginal swabs have been regularly taken three times a week for seven months on twenty six adult female gibbons. Of these animals, menstrual bleeding has occurred in only twenty gibbons. The duration of bleeding and the interval between menstrual periods have been extremely irregular and varies from 7 to 173 days. None of the gibbons in the study have regular cycles. A detailed listing of these findings is shown in Table 1. In order to determine if a typical menstrual cycle could be induced in gibbons as it is in humans by the administration of birth control pills for a 20 day period, a commercial preparation (C-Quens) consisting of 40 mcg. of mestranol was given orally for 15 days and immediately followed by 5 days of 40 mcg. of mestranol plus 1 mcg. of chloromadinone acetate to twelve female gibbons selected randomly from the study group. Menstrual bleeding occurred uniformly in all treated animals on 4th to 5th day following conclusion of this treatment. Because ovulation occurs no earlier than the 11th day following the commencement of menstrual bleeding in other primates that have been studied extensively, the treated group was studied closely from the 11th to the 25th day of the cycle. Laparotomies were performed to directly observe the ovaries for evidence of ovulation or formation of corpora lutea and endometrial biopsies were taken to correlate ovarian findings with histological changes in the endometrium. Rectal examinations were made of the uterus and ovaries before surgery so findings could be confirmed by direct observation later. Of the ten animals examined, only one showed signs of having recently ovulated when its ovaries were examined on day 22 following commencement of menstrual bleeding; there were no signs of a corpus luteum. One other gibbon examined on day 22 had the right ovary that contained what appeared to be a mature follicle. This follicle was distinguished from the other follicles by its large size and good definition. Ovarian abnormalities were observed in two gibbons from this group. Gibbon B-30S had ovaries that were extremely difficult to find and when they were finally located were deeply embedded in large masses of fat. This finding was very much in contrast to the findings in the other animals whose ovaries were exceedingly simple to locate and observe. Both ovaries of gibbon B87 contained very large cysts which occupied about 80% of the mass of each ovary. The cysts had diameters that exceeded 1 centimeter and contained a clear fluid which was easily aspirated by syringe. The ovaries of the remaining gibbons appeared to be quiescent and each one contained a large number of developing follicles, none of which appeared to be mature; no corpora lutea were found. The overall impressions

from this series of observations is that the drug treatment failed to initiate a regular ovarian cycle as had been hoped, since ovarian activity appeared to be negligible. Histopathological examinations of the uterine biopsies obtained at the same time the laporatomies were conducted verified these findings and in only one case could evidence be found that secretory endometrium was developing although even this finding was not characteristic of the true secretory phase.

Experience gained in performing rectal examinations in the study described and during monthly pregnancy exams has shown that it is possible to accurately delineate the shape and consistency of the uterus and in some cases to palpate the ovaries. It is felt that ovarian palpation may be developed to a higher degree as experience in performing these rectal examinations increases. The vagina tends to be either flaccid or firm in consistency and the body of the uterus is consistently firm and from 10–15 mm. in diameter. The uterus is located immediately at, or slightly anterior to the pelvic inlet. The ovaries when it is possible to palpate them, may be located immediately adjacent to either side of the body of the uterus. It is not possible at this time to describe the changes that may occur in the uterus during early pregnancy because there are currently no pregnant gibbons in the colony. It is a simple matter to diagnose pregnancy in late gestation but abdominal palpation and other methods may be used during this time that are simpler and equally reliable.

Histological examination of the endometrial biopsies and Papanicolaou smears taken on five gibbons at 45 day intervals for an extended period of time showed no sign of changes indicating that ovulation had occurred. These observations continue to support those findings described in the last annual report.

During the relocation of the gibbon bleeding colony from Prabuddhabaat to Bangkok in January it became necessary to put the breeding animals in small cages because it was not possible to move the large breeding cages to Bangkok at the same time. During this time matings were maintained in the large 48" by 48" by 24" cage that is used to house experimental animals. From direct observations made by the animal caretakers, this arrangement did not appear to interfere with normal mating behavior. Because of the availability of breeding animals and the desirability of mating them, it was decided to try to use the smaller cages for the mating of new pairs. As is common practice in mating other animals, females were brought to the male cage for mating. Of the six matings that have been made in this manner, only in one case has it been necessary to separate animals because of incompatibility. In several cages copulation occurred almost immediately between animals that had never before been mated and in no case have any animals been seriously injured or required medical treatment. Although the number of offspring that will be produced from this type of mating remains to be determined, it seems that arranging compatible matings is not a significant problem.

The semen of eleven male gibbons has been regularly examined once a week. Semen samples are collected by electroejaculation from animals that are first given Serynlan (Parke Davis) for immobilization. The following is a description of the methods used to evaluate each semen sample. The results of these examinations in six animals are listed in Table 2; they represent the average values of 25 consecutive weekly samples.

a. Semen Morphology:

1. William's Stain preparation. Carbol Fuchsin is used for examining spermatozoa directly by this method. Abnormal spermatozoa may also be observed with this preparation.
2. Nigrosin staining. Nigrosin is used for observing protoplasmic droplets that usually occur at the midsection of the sperm cell's tail.
3. Hematoxylin—Eosin staining or Cell staining. The stain is used visualize pathogenic cells in the semen such as leukocytes and bacteria.
4. Giemsa stain. This stain is also used for examining sperm morphology.
5. Formal—Saline. A formal—saline solution is used for preserving and suspending spermatozoa for examination under a phase contrast microscope.

b. Semen Volume: Volume is determined with a graduated pipette.

- c. Semen Density: The density of spermatozoa in a drop of semen placed on microscope slide is subjectively appraised under low power magnification and graded as very thick, thick, or thin.
- d. Mass activity: Mass activity is subjectively evaluated under low power magnification and graded as follows:
- 0—spermatozoa are immotile
 - 1—stationary or weak rotary movements are exhibited by spermatozoa
 - 2—oscillatory or rotary movements and fewer than 50 percent of the spermatozoa are in progressive motion with no waves of eddies
 - 3—progressive rapid movement of spermatozoa, with slowly moving waves and eddies (usually 50 to 80 percent of the spermatozoa must be progressively motile to produce wave and eddies)
 - 4—vigorous, progressive movement with rapid and abruptly forming waves and eddies, indicating about 90 percent motile spermatozoa
 - 5—very vigorous forward motion, extremely rapid waves and eddies, indicating about 100 percent motile spermatozoa
- e. Motility: Motility is subjectively evaluated under the medium power objective with a cover glass placed over a drop of semen. It is expressed as the percent of spermatozoa that are motile.
- f. Concentration: Concentration is determined by 2 methods:-
1. Spermatozoa are diluted with 5% sodium bicarbonate and 1% Phenol and counted like red cells using Hemocytometer and multiplying by 10,000. The result is the number of spermatozoa in 1 cubic millimeter.
 2. Spermatozoa are diluted with distilled water and then counted by using the Burker Chamber method.
- g. pH of Semen: Standard pHyrion paper is used to determine pH of the semen.

SUMMARY

Continued observations in female gibbons support earlier findings that menstruation occurs irregularly. In addition it is likely that the menstrual cycle is frequently interrupted by extended periods of time when the ovaries are quiescent and the uterine mucosa remains in the early proliferative stage. The factors that precipitate ovulation and the events that characterize the reproductive cycle in other animals continue to be unknown in the gibbon, although the birth of gibbons in the colony proves they do exist. Information accumulated from males shows that gibbon semen is morphologically and quantitatively indistinguishable from what is considered normal in other primates. These observations have been considered in the development of the current mating program in which physically acceptable females are mated only after they experience two periods of menstrual bleeding separated by an interval of no more than 40 days. Males are used as breeders only after a thorough physical examination shows them to be free of physical defects and at least two examinations show that semen values are within normal ranges. Mated females are examined rectally at the end of each month to determine if conception has occurred in which instance the females are removed from the males. This system has been operational for only a short period of time and it is therefore not possible to assess its effectiveness.

Table 1 Occurrence of Menstrual Bleeding in 20 Gibbons Measured in Days.

Number of Animals	1st Time of Bleeding	Interval	2nd Time of Bleeding	Interval	3rd Time of Bleeding	Interval	4th Time of Bleeding	Interval	5th Time of Bleeding	Interval	6th Time of Bleeding
B 4	1 (1)	49	50 (1)								
B 7	1 (3)	9	10 (1)	50	60 (5)	93	153 (1)				
B 30 S	1 (1)	23	24 (1)	21	45 (3)	15	60 (1)	50	130 (1)	39	169 (1)
DZ 1											
B 9	1 (1)	135	136 (1)								
B 85	1 (1)	45	46 (3)	42	88 (3)	53	141 (3)				
B 87	1 (1)	60	61 (2)								
B 50	1 (1)	40	41 (1)	39	80 (1)	12	92 (1)	12	104 (3)	35	139 (1)
B 86	1 (1)	23	24 (1)	8	32 (1)	11	43 (1)				
B 70	1 (1)										
B 25											
B 57	1 (4)	32	33 (1)	18	51 (26)						
B 88	1 (3)	7	8 (3)	7	15 (3)	21	36 (1)	68	104 (4)		
B 11	1 (3)	21	22 (3)	52	74 (1)						
B 37	1 (1)	112	113 (1)								
S 90											
S 84	1 (1)	78	79 (1)								
S 86	1 (3)	28	29 (1)								
P 2	1 (3)	39	40 (1)								
P 7	1 (3)	25	26 (1)								
S 2	1 (1)										
B 51	1 (1)	10	11 (1)	173	184 (1)						

(Figures in parenthesis indicate minimal duration of bleeding in days)

Table 2. Average Gibbon Semen Values

Animal Number	Volume ml.	Density	Mass Activity	Motility %	pH	Concentration per mm ²	Nigrosin Staining		Cell Staining
							Prox. %	Dist. %	
DZ-2	0.3	Thick	++++	86	7.0	1,682,740	2	—	Neg
S-98	0.24	Slightly thick	++	51	7.1	10,82,058	2	—	Neg
B-12	0.13	Slightly thick	+++	59	7.1	1,426,870	1	—	Neg
B-8	0.2	Slightly thick	+++	64	7.2	1,004,286	2	1	Neg
B-21	0.12	Thick	++	46	7.1	1,421,631	1	1	Neg
B-24	0.19	Thick	+++	73	7.0	2,472,727	1	1	Neg