Gibbon Menstrual Cycle and Breeding Study

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PURPOSE: In this study the reproductive cycle of the female gibbon and semen of the male gibbon is characterized and related to other physical parameters of breeding performance with the ultimate goal of obtaining reproduction of the gibbon in a laboratory environment.

DESCRIPTION: During this report period continuing emphasis has been placed on determining the length and characteristics of the menstrual period, the physical characteristics of gibbon semen, and in the effect of combinations of hormones on inducing ovulation in the female. Also during this report period male and female pairs of gibbons were reestablished in eight large outdoor cages that were moved to Bangkok for the purpose of allowing natural mating.

PROGRESS: Data characterizing the female menstrual cycle was obtained by vaginal swabs taken three times a week; in those females that exhibited some degree of regularity swabs were taken seven days a week. At the same time the transverse width of the everted vulva was measured to determine if changes that occurred, if any, were associated with menstrual bleeding. Data from the twenty-nine gibbons that were examined in this manner is shown in Table I. Of interest is the variability in the duration of the menstrual flow and the intervals between menstruation. The variability in bleeding is probably largely artefactual since swabbing only three times a week does not yield accurate results. However, information obtained from swabs taken seven days a week indicates that bleeding ordinarily lasts from three to four days. The variability in the length of the menstrual cycle is consistent with observations made in the last annual report. These intervals are graphically portrayed in figure 1 and show an average interval of approximately 18 days, a period of time considerably less than what would be expected from information collected on other primates. When compared to various times of the year there seems to be no relationship between the occurrence of menstruation and the season. Measurements made on the transverse diameter of the vulva show that in nonpregnant animals the degree of eversion can vary from none at all to three centimeters. Eversion seems to be most pronounced two to three days before bleeding; during bleeding eversion decreases. However, the degree of variability in this finding is so great that it cannot be considered a reliable indicator for determining the stage of the menstrual cycle.

Examination of gibbon semen was continued using the same techniques described in the last annual report. The semen samples were collected from sedated gibbons by electroejaculation using 5 to 15 volts A.C. 50 cycle current and a stimulus of 1 to 2 seconds applied at intervals of 2 to 4 seconds. The results of the semen examinations are presented in Table 2 and do not differ significantly from those reported last year. In an effort to develop the capability for performing artificial insemination an experiment was designed to determine what type of semen expander was the most suitable for diluting and preserving the small volumes of semen collected from the gibbons. Four expanders, egg yolk and dextrose, egg yolk and saline, heated milk, and human seminal plasma, were used in the experiment. The 0.2 to 0.3 ml. of collected semen was diluted with 1 ml. of the expander being examined and kept for varying lengths of time in a 37°C. water-bath. At 30 minute intervals slides were prepared from the diluted specimens and examined for specific activity, motility, and viability. The results shown in Table 3 show that the most suitable expander of those examined is the egg yolk–normal saline solution.

A necessary prerequisite to successful artificial insemination is determining when ovulation in the female occurs. Because of the extreme irregularity in the menstrual cycles of the females studied in the colony, the use of exogenous hormones to induce ovulation appeared to be a practical alternative to using biological indicators. As reported in the last annual report it is possible to regulate stages of the menstrual cycle in a gibbon by giving a course of treatment with progesterone or related compounds. However, as laparotomies and direct observation of the ovaries have shown, it is not possible to predict the time that ovulation would occur with this method. A more complex method used to induce ovulation in macaque species was evaluated in four gibbons that had been cycling at regular intervals. For five days each animal was given 5 mg. of progesterone intramuscularly, then 1 mg. follicle stimulating hormone (Anteron, Schering) intramuscularly for the next four days, and finally 500 international units of human chorionic gonadotrophin (Pregnyl NV. Organon) intramuscularly. A laparotomy was performed on each gibbon in succession at 10 hours, 14 hours, 18 hours and 22 hours following the last injection; times chosen to bracket the ovulation time induced in macaques. The ovaries of these animals all showed multiple follicle development and although the follicles were mature enough to rupture with even the most delicate manipulation there was no evidence that any had ruptured spontaneously.

Eight pairs of gibbons have been mated for approximately six months in the large 20 foot by 20 foot cages moved to Bangkok from Prabuddhabat. The females at first were examined rectally for pregnancy at monthly intervals. In addition, vaginal swabs were taken from the females for the menstrual cycle study and weekly semen samples were being collected from the males. Because none of the animals became pregnant over a period of approximately five months a decision was made to limit the handling of them to only once each quarter and largely eliminate the excitement and trauma that is unavoidable when these animals are restrained. Since that time rectal exams have been performed each quarter in addition to the collection of blood samples for the leukemia screening program, physical examination, and administration of anthelmintics and other necessary treatment. There have been no pregnancies diagnosed.

Table 1. Results of Vaginal Swab Examinations

Gibbon #	Bld_1	Int	Bld_2	Int	Bld_3	Int	Bld_4	Int	Bld_5	Int	Bld_6	Int	Bld_7	Int	Bld_8	Int	Bld_9	Int	Bld_{10}	Int	Bld_{11}	Int	Bld_{12}	Int	Bld_{13}	Int	bld_{14}	Int	Bld_{15}	Int	Bld_{16}	Int	Bld_{17}
B–66–S	3	14	1	47	1	23	1	15	1	15	1	16	1	15	1	21	1	17	3	15	1	15	1	17	1	14	3	18	11	13	3	42	3
B–85	1	44	3	44	2	69	3	47	3	28	1	29	1	21	1	15	1	18	1	29	1	24	3	6	3	13	3	17	3	35	1	14	
F–7	1	32	1	13	1	143	1	18	1	17	3	27	1	27	1	17	3	31	1	37	1	16	1										
S–2	3	35	3	11	1	76	1	15	3	16	6	14	3	13	3	16	3	14	3	11	3												
B–86	1	22	3	99	1	79	1	25	1	20	1	35	1	15	1	29	3	18	1	23	1												
B–57	1	29	3	16	6	36	1	3	9	33	1	3	1	87	3	38	1	23	3														
B–30–S	1	69	3	8	1	20	1	19	1	52	1	23	1	10	6	17	3																
B–11	1	20	1	75	1	19	1	22	1	18	1	27	1	13	1																		
B–88	2	21	1	71	1	79	1	18	1	79	3	11	3	57	3																		
B–7	1	68	1	107	3	46	1	24	1	21	3	25	8	40	1																		
B–87	1	79	1	81	2	42	1	31	1	22	1	63	1	116	1	33	D																
B–10	1	21	3	17	3	16	1	26	1	37	1	11	1	D																			
B–70	1	25	1	48	3	3	1	366	3	6	1																						
S–96	1	28	1	25	1	32	1	8	1	81	1	38																					
B–9	1	41	1	177	1	17	1	117	1																								
B–50	1	35	1	18	1	21	1	33	1	D																							
B–25	3	38	1	9	1	38	1																										
P–2	1	33	1	11	1	4	1																										
B–14–S	1	228	3	18	1																												
B–4	1	64	1	110	1																												
DZ–1	1	122	1	44	1																												
S–90	1	165	2	10	1																												
B–51	1	96	1																														
B–37	1	99	1																														
S–95	1	177	3																														
P–12	1	17	1																														
S–84	1	107	1	D																													
S–10	–																																
S–62	N	–	D																														

Bld_1, Bld_2, Bld_3,... = The first, second, third, time of menstrual bleeding. (The first day of bleeding to the last day of bleeding.)
Int = Time interval (from last day of bleeding to the next first day of bleeding)
D = Dead

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	1,082,058	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–46	0.19	Thick	+++	73	7.0	2,472,727	1	1	Neg.
S–72	0.24	Thick	++	Fair	7	524,000	0.2	0.2	–
S–76	0.1	Thick	++	Good	7.2	247,000	Present	–	–
S–53	0.1	Thick	++	Bad–absent	7.4	116,300	Present	Absent	–
S–74	0.1	Thick	++	Fair	7.1	566,000	Present	Absent	–
B–83	0.1	Thin	+	Bad–absent	7	no spermatozoa	–	–	–
B–18–S	0.1	Thin	++	Bad–absent	7.1	36,000	Absent	Absent	–

Table 3. Gibbon Semen Survivability in Various Expanders (% surviving)

Egg yolk/dextrose	50	40	20	10	<10	0
Egg yolk/saline	60	40	37	33	30	0
Milk	60	20	0			
Human seminal plasma	0					
	5 min.	30 min.	60 min.	90 min.	120 min.	overnight

FIG. I FREQUENCY OF MENSTRUAL BLEEDING RELATED TO THE INTERVAL OF TIME BETWEEN MENSTRUAL PERIODS.