STUDY REPORTS

2. Title: Gibbon Menstrual Cycle and Breeding Study

Principal Investigator: William E. Vick, CPT, VC

Associate Investigators: Richard D. Buchanan, CPT, MC
James D. Pulliam, MAJ, VC
Peter K. Iber, MAJ, MSC

Assistant Investigators: Jack S. Stanton, MAJ, VC
Prayot Tanticharoenyos, DVM

Objective The objective of this study is to obtain reproduction of the gibbon in a laboratory environment and to study menstrual cycles of the female gibbon.

Description The menstrual cycle of the female gibbon in captivity was studied by examination of vaginal smears, endometrial biopsies, basal body temperature variations and urinary levels of estrogens. The possibilities of altering or regulating the menstrual cycle by the use of gonadotropins was investigated. Semen from adult male gibbons was examined and evaluated.

Progress Observations of External Genitalia, Vaginal Mucosa and Menstruation in female gibbons:

Daily recordings of the appearance of the external genital and vaginal mucosa and signs of menstruation were made on 4 adult individually caged female gibbons for periods ranging from 5 months to 1 year.

Gibbon B—58 had a small everted vulva during 5 months of observations. No menstruations were noted nor were there any changes in appearance of the vulva or vaginal mucosa. This gibbon died following anesthetization and subsequent histological examination of the ovaries showed developing and atretic follicles, but no signs of previous ovulation.

Gibbon B—70 was observed for 5 months during which time no menstruation was noted and no changes were observed in the appearance of the external genitalia. Vaginal smears of this gibbon showed repeated atrophic readings and observations were discontinued. The vulva of gibbon B—42 changed from being slightly everted and turgid to highly everted and turgid after four months of observation, however no natural menstruations have occurred during a year of observation.

The appearance of gibbon B—30's vulva changed after two months of observation from being very everted and turgid to being only slightly everted and turgid. This gibbon had a menstruation on 31 October. 1967. and two subsequent menstruations at twenty five and forty day intervals. Each menses lasted three to four days with a scanty discharge being observed during the second and third days. On other days blood was observed only upon flushing the vagina with normal saline solution. No further menstruations have been observed in this gibbon.

Daily Body Temperatures

Daily body temperatures were taken for eighty days on three gibbons (B—30, B—42 and B—66). The temperature readings obtained were directly related to the activity of the gibbon prior to removal

from the cage and the excitability shown by the gibbon during handling on a particular day. Even though the animals selected for temperature determinations were considered to be the most docile in the colony and were handled in a manner to cause the least possible excitement, daily temperatures fluctuoted greatly and were of little or no value in determining stages of the menstrual cycle.

Urinary Estrogens, Pap's Stain of Vagina Smears and Endometrial Biopsies

Urinary estrogen determinations of twenty—four hour urine specimens collected every other day for two months were completed using gibbons B—30 and B—42. Estrogen levels were extremely variable from day to day and no clear data regarding the gibbon estrous cycle could be derived from these determinations.

Vaginal smears were taken every other day along with the urinary estrogen study. Pap smears were classified according to their stage in the estrus cycle and 100 cells per smear were classified by the degree of cellular maturation attained at the time of exfoliation. Cells in the Maturation Index (M.I.) were classified as parabasal, intermediate, or superficial cells.

One of the gibbons, B—30, had two cyclic increases in the number of more mature exfoliated cells. These increases were spaced 26 days apart. Since then neither this animal nor any of the others have shown any cyclic change in Maturation Index. The pattern has been short duration increases and decreases in the number of superficial cells but not regular cyclic changes. These M.I. changes appear to be in response to variations in estrogen levels since none of the animals have shown any definite indications of progesterone stimulation.

Endometrial aspiration biopsies were taken twelve times from gibbons B—66, B—42 and B—30. None of the biopsies showed indications of ovulation, such as subnuclear vacuoles, nor did any have secretory endometrium of the progestational phase. However, all did show indications of various levels of estrogen stimulation and were classified as proliferative phase.

Effect of Injectable Gonadotropins

An attempt was made to determine the effect of injectable gonadotropins in the estrous cycle in gibbons and examine the possibilities of altering or stimulating the cycle in this manner. Gibbons DZ—1 and B—42 were given 5 mg. of Follicular Stimulating Hormone (FSH) intramuscularly for 9 days. One mg. of Luteinizing Hormone was administered intravenously on days 6 thru 9. The percent of superficial cells on Pap smears increased on both gibbons and reached their highest levels on the fifth day of drug administration. DZ—1 menstruated 4 days after the hormone administration and B—42 menstruated 7 days after the hormones were given. The ovaries of both gibbons became enlarged, but slowly regressed in size starting 5 to 10 days after drug administration.

Evaluation of the Male Breeder Gibbons

In October and November of 1967, the semen of all the male breeder gibbons at Prabuddahbaat was evaluated on three occasions. Semen was obtained by electroejaculation and standards of evaluation included approximate amount per ejaculate, appearance, motility, number per cubic milimeter and the percent normal on a sperm smear stained five minutes with 0.25% aqueous basic fuschin. Since the evaluation was completed gibbon B—8 has become a father of an infant gibbon born December 21, 1967, and two other males, B—12 and B—46, have mates which have subsequently becomes pregnant. All three gibbons had sperm which were moderateiy or highly motile. Average semen values for the gibbons are shown in Table 1.

Summary The menstrual cycle of a limited number of captive female gibbons was studied using body temperature, appearance of external genitalia, Pap smears, urinary estrogen levels and endometrial aspiration biopsies as indicies of cyclic stages. Of the gibbons studied only one was observed to have natural menses and these were irregular. Semen from adult male gibbons was collected by electroejaculation and evaluated.

Table 1. Average Values of Gibbon Semen Obtained by Electroejaculation.

Gibbon	Number Sperm per cu. mm.	% Normal Sperm	Motility
DZ—1	1,750,000	70 %	Highly motile
S—98	1,300,000	50 %	Moderately motile
B—8	990,000	60 %	Highly motile
B—12	130,000	63 %	Moderately motile
B—21	20,000	Insufficient number	Slightly motile
B—40	320,000	30 %	Slightly motile
B—46	220,000	60 %	Highly motile
B—56	75,000	20 %	Slightly motile
B—61	3,090,000	52 %	Moderately motile
B—67	2,190,000	50 %	Highly motile
B—72	2,150,000	13 %	No motility
B—73	445,000	66 %	Moderately motile
B—77	1,350,000	50 %	Highly motile
B—78	2,115,000	50 %	Slightly motile
B—80	20,000	50 %	Slightly motile
B—82	990,000	56 %	Highly motile
B—83	20,000	Insufficient number	No motility