

RESEARCH REPORT

Growth and Reproductive Parameters of Bonnet Monkey (*Macaca radiata*)

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ABSTRACT. The present paper summarizes some of the important biological and physiological data recorded over a 30-year period on the biology of bonnet monkeys in captivity. Data on sexual maturity, menstrual cyclicity, general behaviour, endocrine profile, reproductive physiology, gestation, parturition, postpartum amenorrhoea in the female, and sexual maturity, hormone profile, and seasonal variation in sperm count of the male monkeys are presented. In addition to the biological values, weights of selected organs, vertebral and dental pattern are also presented. Menarche occurred at an age of 36 ± 4 months and the first conception in the colony occurred at an age of 54 ± 4 months. The average menstrual cycle length was 28 ± 4.3 days. Majority of monkeys did not cycle regularly during March–June during which the temperature reached a peak. The pregnancy index of the colony was 80% with controlled breeding. The gestation period was 166 ± 5 days with 6–7 months postpartum amenorrhoea. Males attained sexual maturity by the age of 6–7 years and exhibited the characteristic nocturnal surge of serum testosterone at this age and sperm concentration ranged from 116–799 millions/ejaculate.

Key Words: Bonnet monkey; Reproduction; Endocrine physiology; Hormone.

INTRODUCTION

India has 18 species of monkeys of which 7 species belong to genera of *Macaca* within the family Cercopithecidae. The most common macaques extensively used as experimental models for a variety of studies all over the world are the cynomolgus monkey (*Macaca fascicularis*) and rhesus monkey (*Macaca mulatta*) (STEINER & BREMNER, 1981; PLANT, 1982). While the rhesus monkey is widely distributed in northern parts of India, the Indian peninsular region (South India) has a different species of macaque known as the bonnet monkey (*Macaca radiata*). It bears a close physical resemblance to *Macaca mulatta* but is relatively smaller and lighter in frame with a long tail and a bonnet of long dark hairs that radiate in all directions from a whorl on its crown. They breed throughout the year and are highly resistant to epidemic diseases with an average life span of 20 to 25 years in captivity. Although its use in the fields of immunology, nutrition (SEHGAL et al., 1991; SANDYAMANI, 1992) and experimental pathology has been very limited, in recent times, this species is being increasingly utilized due to certain advantages it possesses over other species of primates, some of which are ease of handling, resistance to disease and ability to breed well under captive conditions throughout the year. During the last three decades the bonnet monkey has been used increasingly for studies in the field of reproductive biology, endocrinology, and screening of contraceptive vaccines. (PRAHALADA et al., 1975; KHOLKUTE et al., 1981; MOUDGAL et al., 1985; MATHIALAGAN & RAO, 1986; MOUDGAL et al., 1988; RAVINDRANATH & MOUDGAL, 1990; RAVINDRANATH et al., 1992). Efforts to employ bonnet monkeys for preclinical testing of contraceptive vaccines

and other drugs have been quite successful. However, very little published literature is available on the biology of bonnet monkeys. The objective of the present paper is to compile our observations recorded in our colony of *Macaca radiata* over a period of three decades. In addition, we have also acquired considerable information on the hormonal profiles of both male and female bonnet monkeys under captivity and the present report is a summary of all these findings. The data will be of paramount importance in carrying out biomedical investigations as the bonnet monkey is a surrogate model to human.

MATERIALS AND METHODS

PROCUREMENT OF MONKEYS AND QUARANTINE

The colony was started 30 years ago with a select batch of adult males and females caught from the plantations. Over the years the number of monkeys caught from outside is considerably reduced as we have been able to establish a very active and successful breeding program. The bonnet monkeys were caught from the plantations and forests in the vicinity of Bangalore with the help of trapping cages and were transported to the colony. The animals were treated for any external injuries and transferred to individual cages (0.6m×0.6m×0.6m) provided with a restraining mechanism. During the 2-week prequarantine period, all monkeys were subjected to tuberculin test which was performed by injecting mammalian tuberculin intrapalpebrally followed by a combination course of penicillin and streptomycin (ARYA, 1972). Also faecal samples were evaluated for the presence of parasites. After the prequarantine period the animals were subjected to quarantine for five to six weeks. During this period animals were subjected to physical examination and one more tuberculin test was performed at the end of quarantine. After recruitment into the colony, the animals were weighed, and identification number tattooed on each monkey's chest, blood samples were collected for analysis. In addition, the female monkeys were observed for vaginal bleeding to monitor the cycles.

AGE DETERMINATION OF WILD-CAUGHT MONKEYS

All the wild-caught monkeys were subjected to frequent dental inspections. The details of dentition, wearing out of incisors, molars and dental arch pattern were recorded. These were compared with the observations made in the colony-born monkeys. Based on these data the monkeys were placed in approximate-age groups. In the bonnet monkey the growth of canine teeth continues until the seventh year after birth in the males. The only criteria for determining the age beyond 7 yr of age are comparing the wearing out dental pattern, colouration and the extent of canines in recently caught monkeys with dental pattern and condition of the monkeys of known age group in the colony. This gave an approximate age of the monkeys but the percentage of error is quite high, since the wild-caught monkeys have different food habits during different seasons of the year, while the colony-born monkeys are maintained on a standard feed regimen.

HUSBANDRY

The monkeys were housed in rooms of 3m×4m dimension. A total number of 19 females and 1 male were housed in individual cages of 0.6m×0.6m×0.6m size in each room. In rooms where only males or females were exclusively housed, one member of opposite sex was kept to provide visual stimuli. All the rooms were provided with humidifier ducts which brought in humid air and two exhaust fans were installed in each room. The lighting in the

rooms was regulated with an automatic timer switch. A light dark schedule of 12:12 hr was maintained (SRINATH, 1980). The monkeys were fed on balanced diet (feed pellets) containing 61% carbohydrates, 20% proteins, 5% fat, 1% fibre, 7% moisture and coated with 6% vitamins and trace elements. Over the years we have evaluated the performance of two types of pelleted feed by the monkeys and the animals preferred the extruded pelleted feed which is coated with sugar and trace elements than the hardpressed pellet. Filtered water was provided in stainless steel containers. The average weight of an adult male (6–8 yr) in the colony is 7.5 ± 1.5 kg ($n=150$) and that of an adult female (4–5 yr) is 5 ± 1 kg ($n=90$), and the weight of newborn male is 450 ± 100 g while that of a female is 400 ± 100 g.

Blood samples were collected using vacutainer tubes between 9:00 and 11:00 from unanaesthetized but restrained monkeys. Semen collection was done by electroejaculation by the penile method.

RIA OF HORMONES

Serum concentration of estradiol-17 β and progesterone were estimated as described earlier (RAO et al., 1984). ^3H progesterone (specific activity 96 Ci/mmol) and ^3H estradiol-17 β (specific activity 100 Ci/mmol) were obtained from Amersham International, London, UK. Chorionic gonadotropin in serum was estimated by a specific ELISA using pooled pregnant monkey serum as a standard (CHAKRABORTI & RAO, 1987). The minimum detectable concentration of hormone was 5 ng/ml in the case of mCG and the intra and inter assay variations were 10.3 and 18.5% respectively. In the case of E 2 minimum detectable concentration was 10 pg and intra and inter assay variations were 5.2 and 8.66% respectively. In the case of progesterone the minimum detectable concentration was 20 pg/ml and intra assay and inter assay variation was 6.5 and 10.3% respectively.

BREEDING SCHEDULE

Females were monitored for menstrual cyclicity by vaginal swabbing and also by monitoring serum estradiol-17 β from days 7 to 11 and serum progesterone from 18th to 28th day of the cycle. Two methods of breeding were adopted in the colony. For increasing the colony stock all those monkeys which showed normal menstrual cycle length of 28 ± 3 days were co-habitated with a proven fertile male from days 10–14 of cycle. In these monkeys, serum estradiol 17- β and progesterone were not determined before breeding. However, for studies involving testing of drugs or vaccines having antifertility activity, only those animals which had an extremely regular menstrual cycle and those in which serum estradiol concentration was above 250 pg/ml on day 9 or 10 were cohabitated with proven fertile males. In all cases pregnancy was confirmed by monitoring the serum progesterone on day 18–28 and also by checking for presence of CG in the serum by a specific ELISA.

RESULTS

DENTAL AND VERTEBRAL PATTERN, BIOLOGICAL, ENDOCRINE, AND REPRODUCTIVE PROFILE

The details of appearance of deciduous and permanent teeth in both sexes is presented in Table 1. Other skeletal features recorded during the necropsy are presented in Table 2. The weights of selected organs collected during necropsy for histological studies are presented in Table 3.

Table 1. Appearance of deciduous and permanent teeth in both sexes.

Days of age (range)	<i>N</i>	Appearance of deciduous teeth (either sex)
8 — 11	16	Central incisors
21 — 28	16	Lateral incisors
35 — 56	16	Canines and first molars
77 — 112	16	Formation of complete set of deciduous teeth
Age in months (range)	<i>N</i>	Appearance of permanent teeth
12 — 15	9	First molar
20 — 28	9	All incisors
30 — 36	9	Second molar first premolar
36 — 40	6	Second premolar canine
48 — 72	15	Third molar

Table 2. Skeletal features during the necropsy.

Region	Vertebral column (<i>n</i> =50)
	Vertebrae
Cervical	7
Thoracic	12
Lumbar	7
Sacral	3
Caudal	20
	49
Cervical, thoracic and lumbar	Ventroflexed
Sacral and caudal	Slightly dorsiflexed
RIBS: 12 Pairs	Costae
8	Vertebrosteral
2	Vertebrochondral
2	Vertebrala

SEXUAL BEHAVIOUR

Initially when the newly inducted adult females and males when housed together mating was not observed in the presence of the maintenance staff and animals huddled in a corner of the cage. However, the males accustomed to captivity lifted the tail of female, sniffed and copulated within minutes. Likewise, colony-adopted females presented themselves for copulation immediately after cohabitation. Although colony-born males were not aggressive, they did exhibit mounting behaviour immediately after cohabiting with a female, while the colony-born females were slow in exhibiting sexual response. The data collected on sexual behaviour is from visual observation of both colony-born and wild-caught monkeys over a period of 15 years. The prominent features during mating were smacking of lips, grinding of teeth, lifting the tail of female for sniffing, followed by mating. During the process the female always turned its head with grinding teeth tried to touch the body of the male. A total of 452 pairing were attempted and out of these 405 copulation were observed over a period of 15 years.

MENARCHE AND ADOLESCENCE

In 50 animals of known age, menarche occurred at an age of 36 ± 4 months. Unlike the rhesus monkeys, bonnets did not exhibit sexual skin changes and menstrual flow of blood was the only

Table 3. Organ weights (g) of adult male bonnet monkey ($n=40$) body weight (7.68 ± 0.8 kg).

Brain		85.00 \pm 5.66
Heart		29.33 \pm 5.01
Liver & gall bladder		141.06 \pm 21.91
Pancreas		7.47 \pm 1.59
Pituitary		0.11 \pm 0.03
Thyroid		0.78 \pm 0.16
Adrenal	L	0.7 \pm 0.14
	R	0.6 \pm 0.12
Kidney	L	12.29 \pm 1.74
	R	12.65 \pm 1.79
Lungs	L	19.55 \pm 3.78
	R	15.9 \pm 2.46
Spleen		9.6 \pm 1.95
Testes	L	16.9 \pm 4.8
	R	14.18 \pm 3.6
Gastrointestinal tract		180.14 \pm 22.16

Parathyroids were weighed along with thyroid, gall bladder weighed intact with liver. The capsule was removed from the kidney prior to weighting. The gastrointestinal tract was weighed after discarding the contents by repeated washing.

indication of onset of menses. The first conception in the colony-born females occurred at an age of 54 ± 4 months ($n=20$). Attempts to breed females in early adolescent age were not successful.

CYCLICITY OF ADULT FEMALE BONNET MONKEYS: LENGTH OF MENSTRUAL CYCLE AND MENSTRUAL FLOW

During the year 1981–1995 a total number of 12,850 menstrual cycles of normal females were monitored. This data is from 73 females. However it should be noted that all the females were not observed simultaneously and only those which exhibited regular cycles were included in the results. The average cycle length was 28 ± 4.3 days ($n=73$) and the duration of menstrual flow of blood varied from 2.6 ± 0.5 days. But this could be one day longer since observations were made only once in a day in the morning and the possibility of onset of bleeding in the afternoon could not be ruled out.

The number of cycles recorded from February to June were generally low. This was attributed to be due to summer amenorrhoea. The lowest number of cycles recorded was in the month of May, when the temperature of Bangalore city reached a peak of 38°C . More than 60% of the animals exhibited summer amenorrhoea and irregular cycles, in addition to the increased length of cycles when maintained in rooms not provided with a humidified air circulation. One interesting observation made during the present study was that the presence of an adult breeding male in the room which provided the visual stimulus to females resulted in extremely regular cycles. In addition, by maintaining humidified atmosphere, summer amenorrhoea was almost eliminated thus permitting the most optimal use of monkeys throughout the year. It was also noticed that during the rainy season the total number of monkeys that cycled regularly also increased significantly (RAO et al., 1997).

HORMONAL PATTERN DURING NORMAL MENSTRUAL AND FERTILE CYCLE

Female monkeys that exhibited normal cycle length and menstrual flow were chosen for determining serum concentration of gonadal steroids during follicular phase (Day 7, 8, 9, and 10) and luteal phase (Day 18–28). The concentration of estradiol 17- β increased from day 7 to

10 and the maximal serum concentration recorded before ovulation on day 9 or 10, was ranged from 250 to 900 pg/ml (550 ± 250 pg/ml, $n=2,400$). It was also seen that progesterone was low during the preovulatory phase but gradually increased from day 16 to 20 and reached a peak on day 19 and the concentration ranged from 2–7 ng/ml (5.1 ± 1.9 ng/ml) of serum ($n=2,400$) (RAO et al., 1984).

BREEDING PERFORMANCE

A total number 200 normal cycling female monkeys that had more than 250 pg of estradiol 17- β on day 9 or 10 of 452 cycles were cohabitated with proven fertile breeding males during the period 1981–1995, and this resulted in 365 pregnancies (pregnancy index: 80%). The minimal number of exposures to a proven fertile male required for a female to become pregnant was three for each female monkey, with 60% of the animals becoming pregnant in the first exposure, 16% and 4% in the second and third exposures, respectively. While the percentage of animals that became pregnant when bred randomly was 60–65%, it was as high as 80–85% with animals in which cycles were regularly monitored by checking serum estradiol-17 β levels, on day 9 or 10. It was also observed that unlike the case of several other non-human primates which could be bred after a period ranging from a few months to several years, after recruitment into the colony, *Macaca radiata* could be bred immediately after the quarantine period. One of the important contributing factor for this success is perhaps the fact that these animals are native to the area and thus get acclimatized to the laboratory environment much faster. Several females in the colony have delivered more than five times during their reproductive life span ($n=10$). The age of the oldest female that we have successfully bred in captivity was 15 years ($n=10$).

GESTATION, PARTURITION, AND PARENTAL BEHAVIOUR

The period of gestation in bonnet monkeys ranged from 161 days to 171 days with a Mean and SD of 166 ± 5 days ($n=315$). The male:female ratio of offsprings was 2:1. (Based on data over five years period during which 108 births were recorded out of which 72 are males and 36 females.) Parturition usually occurred during the night. In a few cases where parturition was monitored, the placenta fell off immediately after parturition or it was delayed for a few hours in some cases. Only one case of placental retention was recorded. After the placenta was thrown out, the mother severed the umbilical cord. The mother confined herself to a corner of the cage in the presence of staff. The baby was held firmly to the chest or abdomen. Any attempt to restrain the animal elicited an aggressive response from the mother. Even if the baby was pulled and held for examination, the mother reacted violently, tried to pull the baby back dangerously, thereby injuring the baby. The other monkeys in the room also reacted aggressively making loud noises. A total number of 315 births were recorded between 1981–1995 and 10 monkeys gave birth to 5 babies each during their reproductive life spans.

POSTPARTUM AMENORRHOEA

The babies were weaned at the age of 6 months. The majority of the mothers did not exhibit regular cycles until the baby was weaned and only 10 to 12% lactating animals exhibited cyclicity. However, it was noticed that such animals generally kept the baby away and did not breast feed the baby. After weaning, the animals started cycling within two to three months (MANECKJEE et al., 1976).

MATURITY IN THE MALE BONNET MONKEY

The male bonnet monkey exhibited mounting behaviour at the age of 2.5 yr, when testicles had not yet descended to the scrotum ($n=80$). Between 2.5 yr and 5 yr, there was a marked improvement in the growth appearance of males. The testicles descended to the scrotum and serum testosterone concentrations determined at this age showed a characteristic diurnal rhythm as reported earlier for adult bonnet males (MUKKU et al., 1981). However, they could still only be called young adults at the age of 5, even though they showed a characteristic nocturnal surge of serum testosterone values ranging between 20–40 ng/ml of serum ($n=200$). The minimum age of the male bonnet monkey that was used successfully in the breeding programme was 6 yr ($n=80$) and animals that were generally less than 6 yr were not capable of impregnating. Although, the bonnet monkey is not seasonal in the strict sense like rhesus monkeys in which recrudescence of testes can be seen during the non-breeding season, bonnet monkeys also exhibited changes in sperm count. The lowest count was generally recorded in the month of April and values ranged from 116–228 million/ejaculate ($n=200$). The mean sperm count for adult bonnet monkey during breeding season ranged from 225–799 million/ejaculate ($n=200$) and the volume of the semen varied from 0.5 to 3.0ml. It was observed that it was much easier to electroejaculate a full-grown adult monkey and invariably it was much easier with one that has been used previously for breeding.

It was also noticed that the nocturnal surge of testosterone could be very effectively used to assess the fertility status of a male monkey. In all cases where adult male monkeys which were able to impregnate successfully and which could be used as breeder males invariably had a very good surge of serum testosterone at 22:00 and had a minimum of 5–7 fold difference between 10:00 and 22:00 values. This fold difference between 10:00 and 22:00 levels and 22:00 values decreased as the animals aged and these animals were not very efficient in impregnation.

DISCUSSION

Serious efforts are in progress by the biomedical scientists to minimize the use of animals including non-human primates for *in-vivo* studies and employ animal cell culture as a substitute. However, the use of non-human primates cannot be avoided in some critical areas such as testing of contraceptives, antifertility agents, toxicology of drugs (MOUDGAL, 1981). It is in this context that the judicious use of non-human primates becomes important. Though non-human primate such as chimpanzees and baboons have also been used occasionally in biomedical research, most of the studies have been carried out using macaque species (WILLIAMS & HODGEN, 1982). Of these, *Macaca mulatta* and *Macaca fascicularis* have been extensively used all over the world (WICKINGS & NIESCHLAG, 1980; WEINBAUER et al., 1984). The general features of the menstrual cycle recorded in the case of the bonnet monkeys in the present study are very similar to those recorded for the *Macaca mulatta*, *Macaca fascicularis*, and *Macaca assamensis* which also has 25–35 day menstrual cycle during captivity (WEHRENBURG & DYRENFURTH, 1983). In addition hormonal profiles of estrogen and progesterone during normal cycle and pregnancy in the bonnet monkey are comparable to those reported for *Macaca mulatta* (ATKINSON et al., 1975) and *Macaca fascicularis* (SHAIKH et al., 1978). We have been able to breed the monkeys very successfully in captivity and the first conception in colony born monkeys were recorded at the age of 54 months. This is quite late compared with 48 months reported for rhesus monkey (WILSON et al., 1983). This is perhaps due to outdoor hous-

ing provided for the rhesus in contrast to indoor housing throughout for our monkeys. In contrast to the random breeding, controlled breeding in which serum estradiol levels were monitored prior to mating, improved the percentage of pregnancy significantly. Similar observations have been reported for *Macaca fascicularis* where urinary estrone conjugates were successfully used for detection of optimal mating time (BEHBOODI et al., 1991). Parturition was found to occur only during night after 22:00 and similar results have been reported for *Macaca nemestrina* (GOODLIN & SACKETT, 1983). We have attempted to use both penile and rectal methods and our experience shows that with the bonnet monkey, the penile method was much more consistent and volume of semen was high compared to rectal method. Similar results have been reported by MATSUBAYASHI (1982) using *Macaca fuscata*.

A summary of the endocrine profile of the male bonnet monkey is presented in Table 4. A summary of general characteristics of the reproductive physiology of the female bonnet monkey as well as endocrine profile is presented in Table 5 and 6. Some of the common biological parameters are presented in Table 7. One of the primary criteria in developing an animal model for use in any area of research is whether that species possesses characteristics that allow researchers to obtain information about the specific questions being studied. In addition, availability in sufficient numbers, ability to breed in captivity, resistance to diseases, and ease of handling are other considerations that determine in favour or against the use of a particular animal. Our experience with both male and female bonnet monkeys over the years has shown that these monkeys can be very successfully used for research purposes in areas like reproductive biology, toxicology, screening of medicinal plants etc. The animals are easy to handle, adopt

Table 4. Endocrine profile of the male bonnet monkey ($n=200$).

	ng/ml (\pm)
Range of testosterone levels	0.8 – 20.0
24hr mean	7.2 \pm 1.07
Mean testosterone levels	
During lights on periods 06.00 to 18.00	3.0 \pm 0.4
During lights off periods 18.00 to 06.00	12.31 \pm 1.8
Prolactin	
10:00 level	100 – 130
22:00 level	250 – 275
FSH	
10:00 level	4 – 5
22:00 level	4 – 4.5
LH	
10:00 level	40 – 45
22:00 level	45 – 50
'T'	
10:00 level	2 – 4
22:00 level	16 – 20

Table 5. Reproductive physiology of the female bonnet monkey ($n=100$).

Age at menarche	36 \pm 4.3 months
Length of the menstrual cycle	28 \pm 4.3 days
Period of menstrual flow	4 \pm 1.05 days
Fertile period of mating	day 9 – 14
Duration of pregnancy	166 \pm 5 days
Young per litter	1
Duration of lactational amenorrhoea	6 – 7 months
Ratio of male to female off spring born	2:1
Pregnancy index	
Random breeding controlled	64 \pm 2%
Breeding based on endocrine profile & confirmed ovulation	82 \pm 3%
Age at manopause	17 \pm 1 years

Table 6. Endocrine profile of the female bonnet monkey ($n=100$).

	Days
Occurrence of estrogen surge	9 – 10
Occurrence of LH and FSH surge	10 – 11
Ovulation	11 – 12
Length of the follicular phase	9 – 10
Length of the luteal phase (days between estrogen surge & next menses)	18 – 19
Maximal progesterone level during luteal phase	18 – 21
Sensitivity of corpus luteum to rescue	21 – 23
Implantation occurs approx. (9 days after fertilization)	22 – 24
CG detection by RIA in serum	27 – 50

Table 7. Common biological values ($n=75$).

Chromosome number	$n=21$	
Average food intake per day		
Male	200 – 300gm	
Female	150 – 200gm	
Average water intake per day	300 – 400ml	
Life span (in captivity)	20 – 25 years	
Daily urinary volume	100 – 150ml	
Daily foecal weight	150 – 200gm	
Body temperature (rectal)	100 – 101 °F	
Heart rate (beats/min)	160 – 300	
Respiratory rate (breaths/min)	39 – 60	
Blood pressure	Systolic	159
	Diastolic	127
Pulse	100/min	
Blood pH (plasma)	7.3	
Body weight	Adult male	7 – 9kg
	Adult female	4.5 – 6kg
Body weight at birth	Male	450 ± 100g
	Female	400 ± 100g

easily to laboratory conditions and to the staff who handle the animals. As indicated earlier, the adult animals weigh less compared to the rhesus monkeys and considering both size and weight, the space requirements are also less. It should be noted that during the last 15 years we have recorded a total of 315 live births from 110 females. Out of these some ($n=10$) have delivered more than five times in ten years establishing the efficacy of breeding. It is also pertinent to record that with controlled breeding, where cycles and hormones are monitored before cohabitation with a proven fertile male, the percentage of success was as high as 85% which should certainly help in establishing a self-sustaining colony.

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