J. Biosci., Vol. 10, Number 4, December 1986, pp. 443-452. © Printed in India.

Circadian and circannual changes in the testicular function of adult rhesus monkeys

ATAM SEHGAL, G. F. X. DAVID, A. K. DUBEY, J. S. PRUTHI and T. C. ANAND KUMAR*
Experimental Biology Unit, Department of Anatomy, All India Institute of Medical Sciences, WHO-CCCR in Human Reproduction, New Delhi 110 029

* Present Address: Institute for Research in Reproduction, Jehangir Merwanji Street, Parel, Bombay 400 012, India

MS received 28 October 1985; revised 15 October 1986

Abstract. The endocrine and gametogenic status of the testes were studied in 9 healthy adult rhesus monkeys of proven fertility throughout a one-year period. Testosterone levels were estimated by radioimmunoassay in blood samples collected at 4 h intervals over a 24 h period once a month. Semen samples and testicular biopsies were also examined once a month. A well-defined circandian rhythm was evident in serum levels of testosterone. The rhythmicity was less pronounced in February and September. The 24 h mean levels of serum testosterone were high between the months of August to March and low in the months of May to July. All animals did not uniformly respond to electro-ejaculation in April and May. Semen volume and total number of spermatozoa were maximal between September and March and least from April to August. Testicular biopsies indicated that all stages of spermatogenesis were evident between September and March and the spermatogenic activity was less evident between April and August. The contents of Sertoli cells showed a seasonal cyclicity; they were laden with lipid droplets during April to August when spermatogenesis was quiescent and vacuolated during September to March when spermatogenesis was active. These studies indicate that the testing of contraceptive drugs needs to be restricted to months of September to March in male rhesus monkeys otherwise, it is possible that the naturally occurring reproductive quiscence may be attributed to the effect of the drug being tested. The data accrued from the present studies also provide quantitative information on circulating levels of testosterone which could be used as a reference background while evaluating the contraceptive drug-effects in male rhesus monkeys.

Keywords. Testicular function; circadian and circannual changes; seasonality;rhesus monkey.

Introduction

New drugs developed for the regulation of human fertility are evaluated for their toxicological safety and efficacy in non-human primates because of the close similarities in their reproductive system to that of man (Diczfalusy and Standley, 1972; Prasad and Anand Kumar, 1977; Anand Kumar, 1980). The rhesus monkey is one of the most widely used species of non-human primates for such purposes. However, unlike man, the rhesus monkey exhibits a distinct seasonality in its reproductive performance (Prakash, 1962; Conaway and Sade, 1965; Southwick *et al.*1965; Vandenburg and Vessy, 1968; Michael and Keverne, 1971; Michael *et al.,* 1975; Gordon *et al.,* 1976; Anand Kumar *et al.,* 1980; Nieschlag and Wickings,

1980). These seasonal changes need to be taken into consideration while evaluating the effects of contraceptive drugs in the rhesus monkey.

This communication describes the seasonal changes occurring in serum levels of testosterone and the gametogenic function of the testes in a caged colony of rhesus monkeys. The information is of particular relevance to the preclinical evaluation of new fertility regulating drugs. As far as we are aware, this is the first description in which the endocrine function of the testis and its histological features are described in the same group of monkeys through the year.

Materials and methods

Animals

Nine healthy male monkeys of proven fertility (8-12 kg body weight) were selected from the breeding colony of the Primate Research Facility of the All India Institute of Medical Sciences. The animals were caged individually and maintained under uniform lighting schedule of 14 h (light):-10 h (dark) throughout the year. Animal husbandry methods followed in this facility have been described (Anand Kumar *et al.,* 1980).

Study design

This study was carried out between January and December. The animals were subjected to blood sampling, penile electro-ejaculation and testicular biopsies respectively on the 15th, 17th and 18th of each month.

Estimating circulating levels of hormones

Blood (10 ml per sample) was collected from conscious monkeys from the antecubital or saphenous vein once a month over a 24 h period at intervals of 4 h. Blood sampling was started at 08.00 h and completed at the same time on the following day. Thus, a total number of 7 blood samples was collected from each animal every month for 12 months. Serum was separated and stored at -20°C until levels of testosterone were estimated by radioimmunoassay (RIA) using reagents supplied by the WHO and following methods described in the WHO Method Manual (1981). The inter-and intra-assay variations were respectively 6·4 and 3·6% (n=22). The antisera for testosterone cross-reacted with dihydrotestosterone to an extent of 50% (Puri *et al.,* 1981). The data was subjected to logit-log transformation and statistical analysis was carried out by students *'t'* test (Snedecor and Cochrane, 1975) to determine the significance of differences between day and night levels of testosterone as well as between the 24 h mean testosterone levels during different months of the year.

Semenology

Monkeys were electro-ejaculated and the semen collected in clean vials. The total number of spermatozoa present in the ejaculate was determined as described by Belsey *et al.* (1980). The volume of semen was estimated by transferring the entire

ejaculate to a 10 ml graduated measuring cylinder containing 5 ml of distilled water and reading off the displaced volume of water.

Testicular biopsies

The testicular biopsies were performed at different sites in the following sequence: (i) the cranial end of the left testis, (ii) caudal end of the right testis, (iii) caudal end of the left testis and (iv) cranial end of the right testis. A few pieces of the tissue were immersed in Bouin's fluid, processed for paraffin embedding, sectioned at 6 μm thickness and stained with haematoxylin eosin. Other pieces of tissue were immersed in Karnovsky's fluid, processed, embedded in Araldite, sectioned at 1 μm thickness and stained with toludine blue.

Results

Testosterone levels

A well-defined circadian rhythm was evident in the circulating levels of testosterone (figure 1). The peak value of about 55 nmol.1^{-1} occurred around mid-night and the lowest value of about 17 nmol·l^{-1} occurred around mid-day. This circadian rhythmicity was evident during all the months of the year (figure 2). However, the magnitude of difference between the day and night levels was the least in February and September (figure 2).

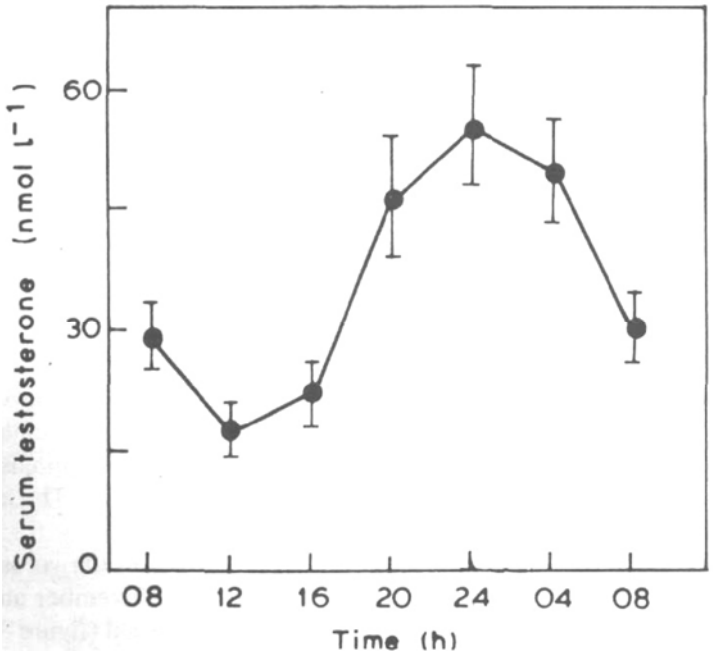

Figure 1. Serum testosterone levels at different times of the day in 9 monkeys studied throughout the year. The lowest level is seen around mid-day while highest level around mid-night. Each point represents the geometric mean (vertical bars indicate 95% confidence limits) of 108 observations.

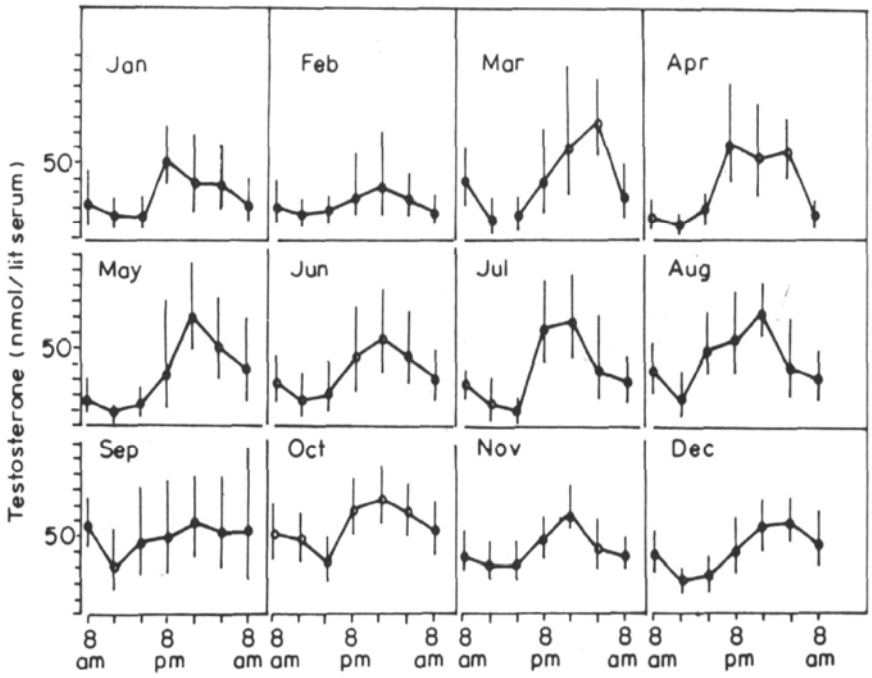

Figure 2. Circadian pattern of circulating levels of serum testosterone in 9 monkeys studied throughout the year. The circadian rhythmicity is evident during all the months of the year. The magnitude of differences between day and night levels are least in February and September. Each point is the geometric mean of 9 observations with 95 % confidence limits indicated in vertical bars.

A comparison of the mean values of testosterone levels in the pooled serum samples collected over a 24 h period showed that significantly high levels occurred during August, September and October and again in March. The values for these months were not significantly different from one another as they were for the other months.

Semenology

All the 9 animals responded positively to the electro-ejaculatory stimulus in January, the first month of the study. Thereafter, one monkey remained totally refractory to the stimulus throughout the year. The number of animals responding to electro-ejaculation also varied between the months (figure 3). The least number of responders were seen in April and May.

Seminal fluid volume was lowest from May to August and the volume began to rise from September onwards to reach maximal levels in November and remained so till February. Thereafter, the seminal fluid volume declined (figure 3). The total number of spermatozoa per ejaculate showed marked differences between individuals. However, mean values were lowest between the months of April to August and during the following months the number of spermatozoa increased to reach peak levels in November and remained so until January (figure 3).

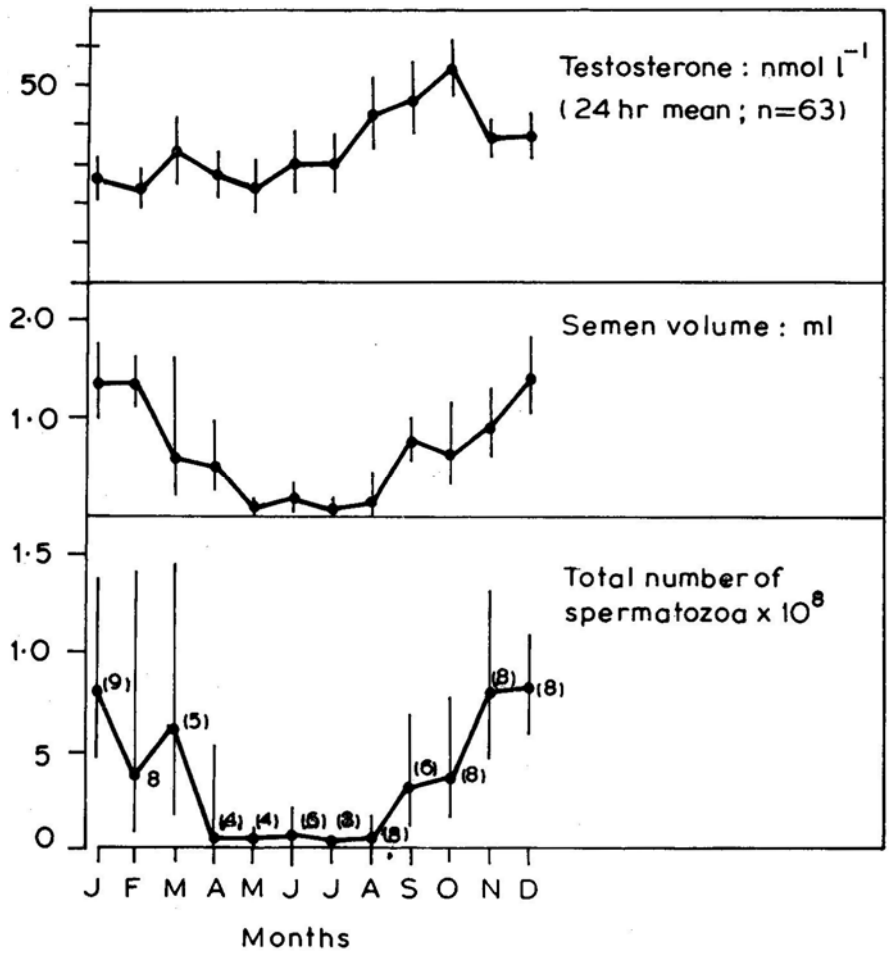

Figure 3. Composite diagram to illustrate the 24 h mean levels of serum testosterone, semen volume and the total number of spermatozoa per ejaculate during different months of the year. Mean levels of serum testosterone for the months March. September and October are not significantly different from one another but are significantly (P<0·001) different from the other months. Seminal volume is the lowest during May. June. July and August in contrast to the other months. The figures in parentheses indicate the number of animals out of the 9 monkeys studied responding to electro-ejaculation during each month. The total number of spermatozoa is the lowest during the months of April to August as compared with the remaining months.

Testicular histology

Marked seasonal changes were evident in the spermatogenic activity through out the year. The seminiferous tubules were large and exhibited all stages of spermatogenesis between September and March. Between April and August there was an obvious reduction in the spermatogenic stages and mitotic divisions (figure 4).

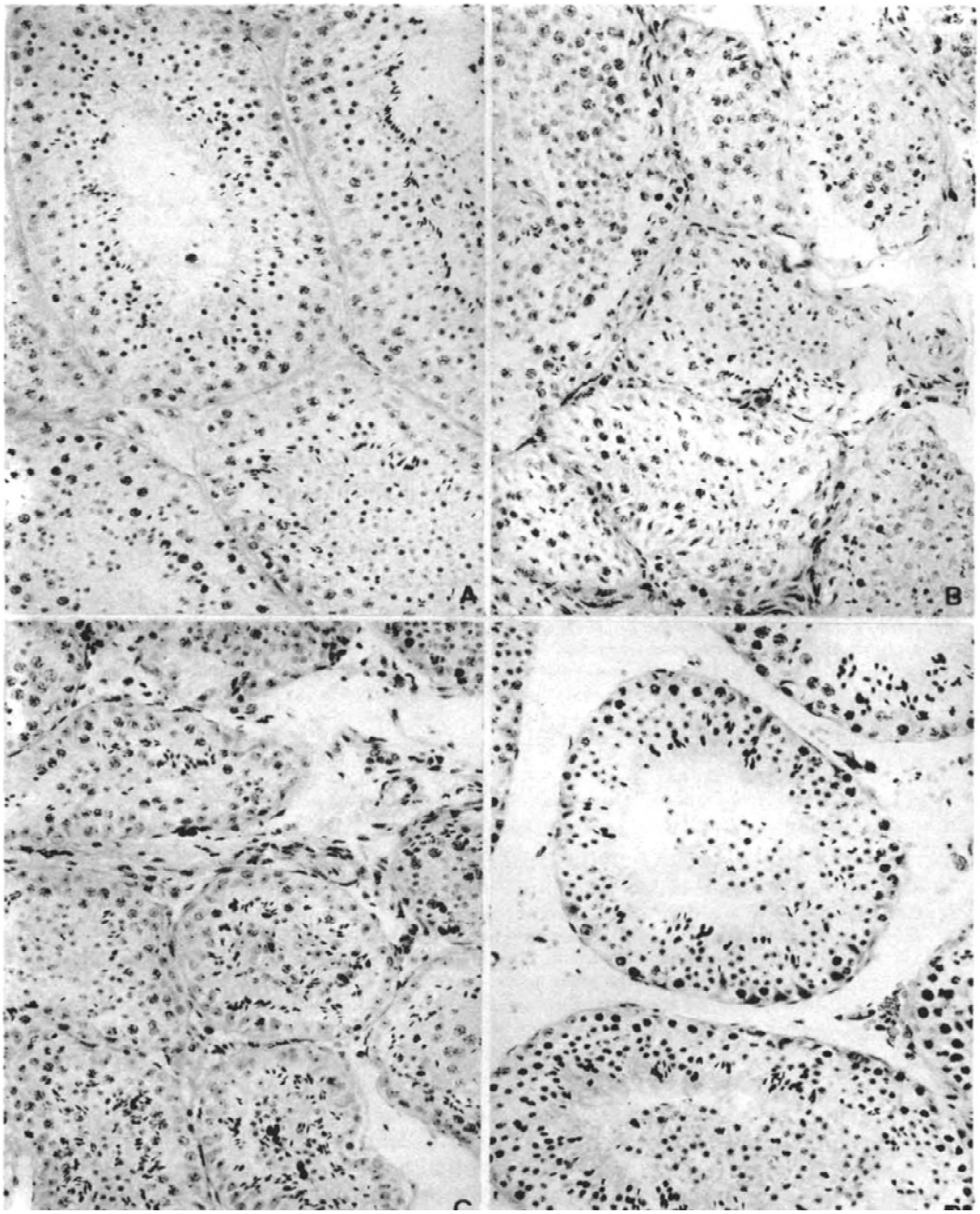

Figure 4. Photomicrographs of testicular biopsies taken in January (A), April (B), July (C) and October (D) and sectioned and stained with haematoxylin-eosin. Spermatogenic activity is low during April (× 320).

Araldite sections of testicular tissues stained with toludine blue showed the presence of metachromatically stained lipid droplets in the Sertoli cells. The Sertoli cells were fully laden with such droplets from April to August when spermatogenesis was quiscent. During the remaining months, when spermatogenesis was active, the Sertoli cells were depleted of their lipid droplet contents and only cytoplasmic vacuoles were evident (figure 5). Thus, the depletion of lipid droplets in the Sertolic cells was coincidental with the increase in spermatogenic activity.

Discussion

The present studies on a group of proven fertile males kept under captivity confirm earlier reports (Goodman *et al.,* 1974; Michael *et al.,* 1974, Perachio *et al.,* 1977) of the occurrence of a distinct circadian rhythmic pattern in the circulating levels of testosterone in rhesus monkeys. Peak levels occurred around mid-night and the lowest levels during mid-day. Our studies have also shown that although the circadian rhythmic pattern was evident throughout the year, the magnitude of difference between the peak and low levels was less evident during February and September as compared with the other months. This may well be due to variation between individual in which the actual peak levels occurred between 20.00 h and 04.00 h. It may be noted that February marks the end of winter in Delhi and the onset of spring while September marks the begining of autumn. It would be interesting to determine whether the marked individual variations observed in the time at which actual peak levels of testosterone occur during the night is indicative of the diurnal rhythmically undergoing re-entrainment in February and September which marks the begining of summer and winter.

Circadian rhythmicity in circulating levels of testosterone has been reported in a number of primate species including man. In man, peak levels of testosterone occur around 04.00 h (Barberia *et al.,* 1973) or 06.00 h (Sjorberg *et al.,* 1979). The biological significance of such circadian variations in circulating levels of testosterone remains to be determined. There is some evidence, however, to indicate that the abolition of the nocturnal surge of testosterone in the monkey may impair spermatogenesis (Moudgal *et al.,* 1985).

A comparison of the mean values of testosterone observed in blood samples collected over 24 h period during different months of the year clearly indicated that maximal values were observed in August, September and October. These findings are in conformity with earlier reports (Plant *et al.,* 1974; Michael *et al.,* 1975; Gordon *et al.,* 1976; Michael and Bonsai, 1977; Beck *et al.,* 1979; Wickings and Nieschlag, 1980) of a circannual rhythmicity in circulating levels of testosterone.

The number of animals responding to electro-ejaculation varied between the months. The least response (4 out of 9) was observed in April and May. A previous study by Wickings and Nieschlag (1980) revealed that none of their animals responded to electro-ejaculation during the summer months. The poor response during the summer months has been related to the drop in circulating androgen levels during this period (Michael and Wilson, 1974). The volume of the seminal fluid collected by electro-ejaculation was also reduced during the summer months which may be indicative of a deprivation of androgenic support to the accessory reproductive organs.

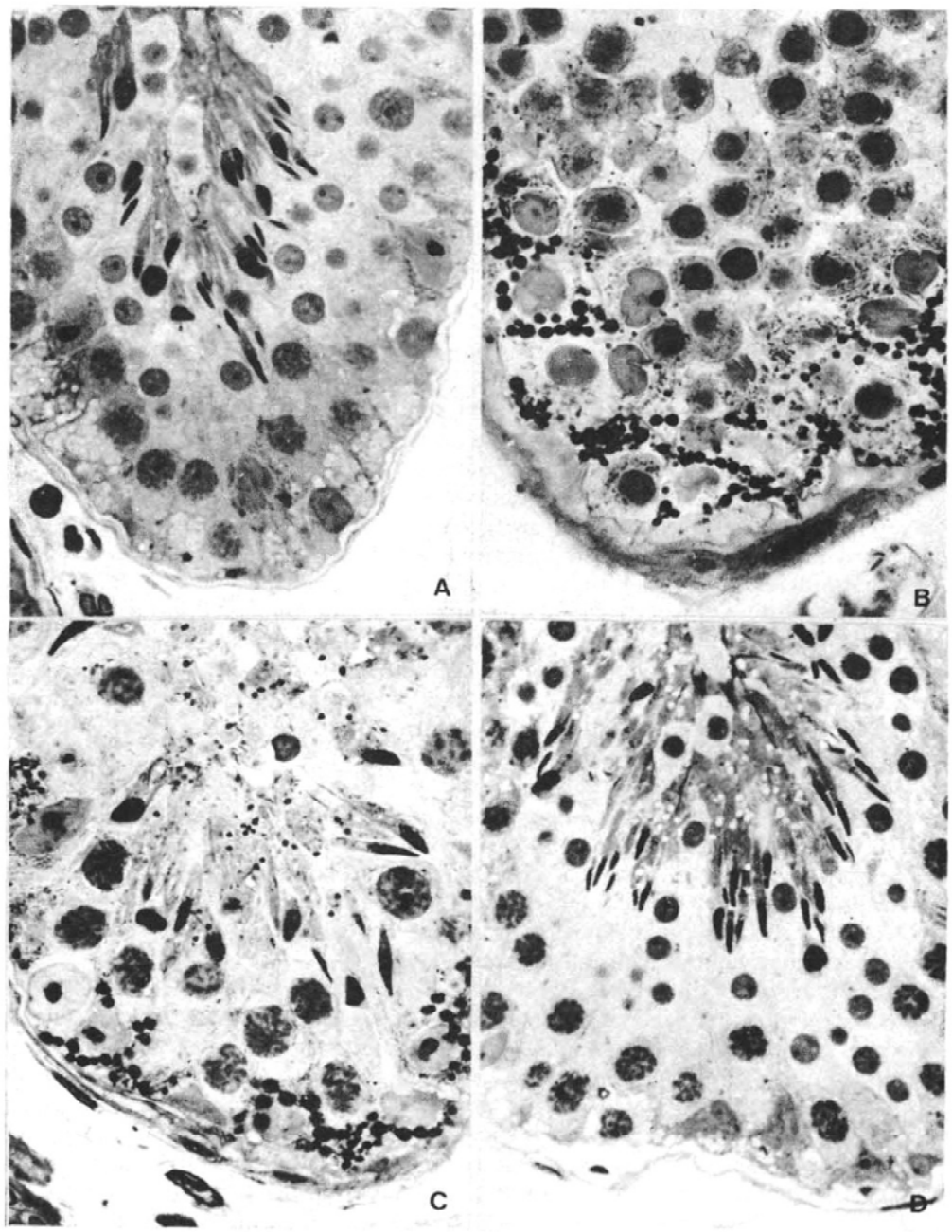

Figure 5. Photomicrographs of araldite sections of testicular biopsies obtained in January (A), April (B), July (C) and October (D) and stained with toludine blue. These photomicrographs illustrate the cyclical changes in the Sertoli cell contents of lipid droplets in relation to the spermatogenic activity. The cells are laden with the lipid droplets when spermatogenesis is quiescent *(e.g.* B and C) and vacuolated during active spermatogenesis *(e.g.* A and D) (× 1200).

Spermatogenesis was quiescent from April to August. In conformity with the reduction in spermatogenic activity, the total number of spermatozoa in the electro-ejaculate was markedly also reduced.

Seasonal changes in spermatogenesis have been reported previously in free ranging rhesus monkeys (Conaway and Sade, 1965). The present studies confirm the occurrence of a similar situation in a caged colony of monkeys. Controlled breeding of rhesus monkeys in our colony has revealed that the maximal number of conceptions occurs during the months of September to March (Anand Kumar *et al.,* 1980) which is coincidental with the period when the volume of ejaculated semen and the total number of spermatozoa are high.

The present studies have, for the first time, revealed a seasonal cyclicity in the contents of Sertoli cells. The vacuolation of the Sertoli cells during the period of heightened spermatogenic activity could be related to the nursing role played'by the Sertoli cells to the spermatids during spermatogenesis.

The data accrued from the present studies clearly suggest that the evaluation of contraceptive drugs on testicular function of rhesus monkeys needs to be carried out between September and March when the testis is fully functional. Studies carried out during the other months would not be meaningful as the testes would be quiescent. Our data also provide quantitative information on circulating levels of testosterone throughout the year which could be used as a reference background while interpreting the drug-induction effects to be evaluated.

Acknowledgement

This work was supported by the World Health Organization, Geneva to the WHO-CCR, New Delhi and All India Institute of Medical Sciences, New Delhi.

References

Anand Kumar, T. C. (1980) *Non Primate Models for Study of Human Reproduction* (Basel: S. Karger).

Anand Kumar, T. C, David, G. F. X., Sharma, D. N., Puri, C. P., Puri. V., Dubey. A. K., Sehgal, A., Sankaranarayan, A. and Pruthi, J. S. (1980) in *Non-Human Primate Models for Study of Human Reproduction,* (ed. T. C. Anand Kumar), (Basel: S. Karger) p. 37.

Barberia, J. M., Giner, J. and Cartes-Gallegos. V. (1973) *Steroids,* **22,** 615.

Beck, W., Hancke, J. L. and Wultke, W. (1979) *J. Endocrinol.,* **83,** 93.

Belsey, M. A., Eliason, R., Gallegos, A. J., Moghissi. K. S., Paulsen, C. A. and Prasad. M. R. N. (eds) (1980) *Laboratory Manual for the examination of human semen and semen-cervical mucus interaction.* World Health Organization (Singapore: Press Concern).

Conaway, C. H. and Sade, D. S. (1965) *Folia Primatol.,* **3,** 1.

Diczfalusy, E. and Standley C. C. (1972) *Proceedings of WHO Symposium,* Karolinska Institute. Stockholm, Acta Endocrinologica. Suppl. 166.

Goodman, R. L., Hotchkiss, J., Karsch, F. J. and Knobil, E. (1974) *Biol. Reprod.,* **11,** 624. Gordon, T. P., Rose. R. M. and Bernstein, I. S. (1976) *Horm. Behav.,* **7,** 229.

Michael, R. P. and Bonsall, R. W. (1977) *J. Reprod. Fertil.,* **49,** 129.

Michael, R. P. and Keverne, E. B. (1971) *J. Reprod. Fertil.,* **25,** 95.

Michael, R. P., Setchell, K. D. R. and Plant, T. M. (1974) *J. Endocrinol.,* **63,** 325. Michael, R. P. and Wilson, M. I. (1974) *Endocrinology,* **95,** 150.

Michael, R. P., Zumpe, D., Plant. T. M. and Evans, R. G. (1975) *J. Reprod. Fertil.,* **45,** 169.

Moudgal, N. R., Rao, A. J., Murthy, G. S. R. C, Neelakanta, R., Banavar, S. R., Kotagi, S. G. and Anand Kumar, T. C. (1985) *Fertil. Steril.,* **44,** 120.

Nieschlag, E. and Wickings, E. J. (1980) in *Non Human Primate Modles for Study of Human Reproduction* (ed. T. C. Anand Kumar) (Basel: S. Karger) p. 136.

Perachio, A. A., Alexander, M., Marr, L. D. and Collins, D. C. (1977) *Steroids,* **29,** 21.

Plant, T. M., Zumpe, D., Sauls, M. and Michael, R. P. (1974) *J. Endocrinol.,* **64,** 403.

Prakash, I. (1962) *Jpn. J. Ecol.,* **12,** 83.

Prasad, M. R. N. and Anand Kumar, T. C. (1977) *Use of non-Human Primates in Biomedical Research,* Indian National Science Academy, (Delhi: Delhi Univ. Press).

Puri, V., Puri, C. P. and Anand Kumar, T. C. (1981) *J. Steroid Biochem.,* **14,** 877.

Sjorberg, B., delaTorre, B., Hedman, M., Falkay, G. and Diczfalusy, E. (1979)*J. Endocrinol, Invest.,* **2,** 131.

Snedecor, G. W. and Cochrane, W. G. (1975) *Statistical Methods,* 6th edition (New Delhi: India Print).

Southwick, C. H., Beg, M. A. and Siddiqui, M. R. (1965) *Primate Behav.,* 111.

Vandenburg, J. G. and Vessey, S. (1968) *J. Reprod. Fertil.,* **15,** 71.

Wickings, E. J. and Nieschlag, E. (1980) *Int. J. Androl.,* **3,** 87.

World Health Organization Collaborating Centre for Research. Reference Services in the Immunoassay of Hormones in Human Reproduction (1981) *Method Manual,* 5th edition.