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Correlation of seasonal changes in sperm output with endocrinological changes in the adult male bonnet monkey, *Macaca radiata*

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Abstract. We have examined the monthly variations in sperm output and attempted to correlate the profiles of endocrine hormones secreted with the sperm counts throughout the year in the adult male bonnet monkey. As previously reported, there was a distinct spurt in sperm output beginning September through December months. A concomitant increase in serum testosterone and prolactin concentrations were also noted during September through November (mid and post-monsoon season). Although there was a marked increase in gonadotropin releasing hormone stimulated testosterone secretion, the peak testosterone concentrations post gonadotropin releasing hormone injection did not vary significantly ($P > 0.05$) throughout the year. Basal serum follicle stimulating hormone concentrations did not vary significantly ($P > 0.05$) during April to June months compared to September-November months. Serum inhibin concentration remained unaltered throughout the year, except in the month of March. The results of this study provide evidence for annual rhythms in prolactin and testosterone secretion and a distinct seasonality in the sperm output of the adult male bonnet monkey, but the pituitary responsiveness to exogenous gonadotropin releasing hormone remains unaltered throughout the year. Because of the existence of seasonality as noted in the present study, future studies which utilize the adult male bonnet monkey as an experimental model need to take into consideration the seasonal effects on reproductive function in this species.

Keywords. Annual rhythms; testosterone; prolactin; GnRH responsivity; male bonnets.

1. Introduction

A distinct seasonality in breeding exists in both wild and captive rhesus and Japanese macaque monkeys (Vanderbergh and Vessey 1968; Riesen *et al* 1971; Michael *et al* 1975; Gordon *et al* 1976; Matsubayashi *et al* 1991). On the other hand, the male bonnet monkey housed indoors appears to breed throughout the year (Srinath 1980). Although previous studies from this laboratory found no evidence for reproductive seasonality in male bonnet monkeys, more recent studies however, have found a distinct decrease in the sperm output during pre and early-monsoon season (March-June) as compared to the mid and post-monsoon (July-November) season (Moudgal *et al* 1992; Aravindan *et al* 1993). Furthermore, DNA flow cytometric analysis of spermatozoa has revealed subtle changes in the quality of sperms analysed during the two seasons. For instance, the ejaculated spermatozoa were more susceptible to induced decondensation in pre/early-monsoon

season as compared to that observed during mid/post-monsoon season (Aravindan G R and Moudgal N R, unpublished data)· Interestingly, it was recently shown from this laboratory (Aravindan *et al* 1993) that specific follicle stimulating hormone (FSH) lack also results in changes in the susceptibility of ejaculated sperm to decondensation, a phenomenon that is quite similar to that observed during pre/early-monsoon season· We hypothesized that the reduction in sperm output as well as its poor quality observed during pre/early-monsoon months could be a consequence of alterations in the endocrine secretory pattern· The purpose of this study was to document the normal patterns of endocrine hormone secretion throughout the year as well as to examine alterations in serum FSH and testosterone (T) concentrations in response to monthly injection of exogenous gonadotropin releasing hormone (GnRH) challenge in adult male bonnet monkeys.

2. Materials and methods

2.1 *Animals*

Sixteen adult male bonnet monkeys weighing 6·8 to 7·4 kg were used in this study. The general care and maintenance of monkeys under controlled photoperiod (12L : 12D; lights off 1800h) have been described earlier (Ravindranath and Moudgal 1987)· Ten of the 16 monkeys were subjected to monthly electroejaculation as reported earlier (Aravindan *et al* 1993).

GnRH was kindly provided by Dr G Bialy of Contraceptive Development Branch, NICHHD, Bethesda, MD, USA. GnRH was prepared as 25(µg/ml stock solution and stored frozen. The same batch of GnRH was used throughout the experiment.

2.2 *Radioimmunoassays*

Serum T was assayed without chromatography as previously described (Rao *et al* 1989). The sensitivity of the assay was l0pg/tube. The inter- and intra-assay coefficient of variations were 11·8 % and 10% respectively·

Serum FSH was measured by a solid phase radioimmunoassay (RIA) as reported previously (Aravindan *et al* 1990). The sensitivity of the assay was 0·4 ng equivalent of hFSH-AFP 4822B/tube. Serum inhibin (INH) concentrations were measured using an NIH kit distributed by the contraceptive Development Branch, NICHHD, Bethesda, MD, USA. The details of the assay standardization from this laboratory has been reported recently (Medhamurthy *et al* 1993)· Serum prolactin (PRL) was assayed as previously reported (Rao *et al* 1989) using human PRL RIA kit kindly provided by the NIH. The procedure provided with the kit, however, was modified to advantage and involved use of a solid phase method as reported earlier from this laboratory for polypeptide hormones (Murthy *et al* 1989). The sensitivity of the assay was 0·4 ng/tube.

The inter- and intra-assay coefficients of variation for FSH, INH and PRL were 14% and 11 % , 8·2 % and 4·6 %, and 10 % and 8%, respectively.

2.3 *Statistical analyses*

The data are represented as mean ± SEM. Student's '*t*' test was used to calculate the significance of difference in hormone concentrations between April-June and

September-November months. The area under curve (AUC) was calculated for each 18 h (overnight) sampling session to determine the nocturnal variations in serum T secretion throughout the year· Peak T and FSH concentrations post GnRH injections were also compared amongst months using the student's '*t*' test.

2.4 *Experimental protocol*

In order to characterize basal hormone secretion, blood samples were collected from three adult male monkeys 1 h before iv administration of 10 μg of GnRH in 1 ml saline once a month from March 91 to April 92. Blood samples were collected at 10-60 min intervals for 3 h after GnRH injection for measuring serum T and FSH to assess the pituitary gland's responsivity. The experiment was conducted between 0800 and 1200 h.

The second experiment was conducted with a view to determine the nocturnal variations in serum T secretory pattern during different months of the year. For this purpose, blood samples were collected from 3 adult unanaesthetized male monkeys every 2h throughout the 18 h period (between 1600 h to 1000 h) during alternate months between March 91 and May 92.

3. Results

Circulating basal serum concentrations of PRL and T were correlated with sperm counts throughout the 12 month period (figure 1). For comparison, mean serum PRL and T concentrations were computed separately for pre/early-monsoon season and mid/post-monsoon season respectively. The pooled mean basal serum T concentration during pre/early-monsoon season was 5·2 ± 2·4 ng/ml and was significantly ($P < 0·01$) lower compared to mid/post-monsoon season (9·5 ± 3·8 ng/ml)· Similarly, the pooled mean basal serum PRL concentration although lower during pre/early-monsoon season but was not significantly different (figure 1; $P > 0.05$) compared to mid/post-monsoon season (14·0 ± 5·3 *vs* 20·5 ± 8 ng/ml). Also shown in figure 1c is the sperm count obtained from 10 male monkeys· A significant increase ($P < 0·05$) in sperm output was observed in October and December months.

The mean basal serum FSH and INH concentrations throughout the 12 month period are depicted in figure 2. Basal serum FSH concentrations were low throughout the year (figure 2) and comparison of pooled mean serum FSH concentrations between pre/early-monsoon and mid/post-monsoon season did not reveal significant differences ($P >0·05$, 2·54 ± 0·9 *vs* 2·28 ± 0·5 ng/ml).

Monthly injection of exogenous GnRH readily elicited significant increase in serum FSH and T concentrations throughout the 12 month period (figure 3). However, the mean peak T concentration observed 40–60 min after each GnRH injection was not significantly ($P > 0·05$) different amongst different months (figure 3). Mean FSH concentration increased less following GnRH injection (figure 3) and the peak FSH concentration post-GNRH was significantly higher ($P < 0·01$) during pre/early-monsoon (April-June) compared to mid/post-monsopn season (Sept-Nov; 4·4 ± 1·3 *vs* 2·90 ± 1·1). For a better comprehension of the data, the peak hormone concentrations in addition to mean basal secretion of each hormone are also shown in figure 3.

The nocturnal serum T profiles representing the highest (Nov) and lowest (Mar)

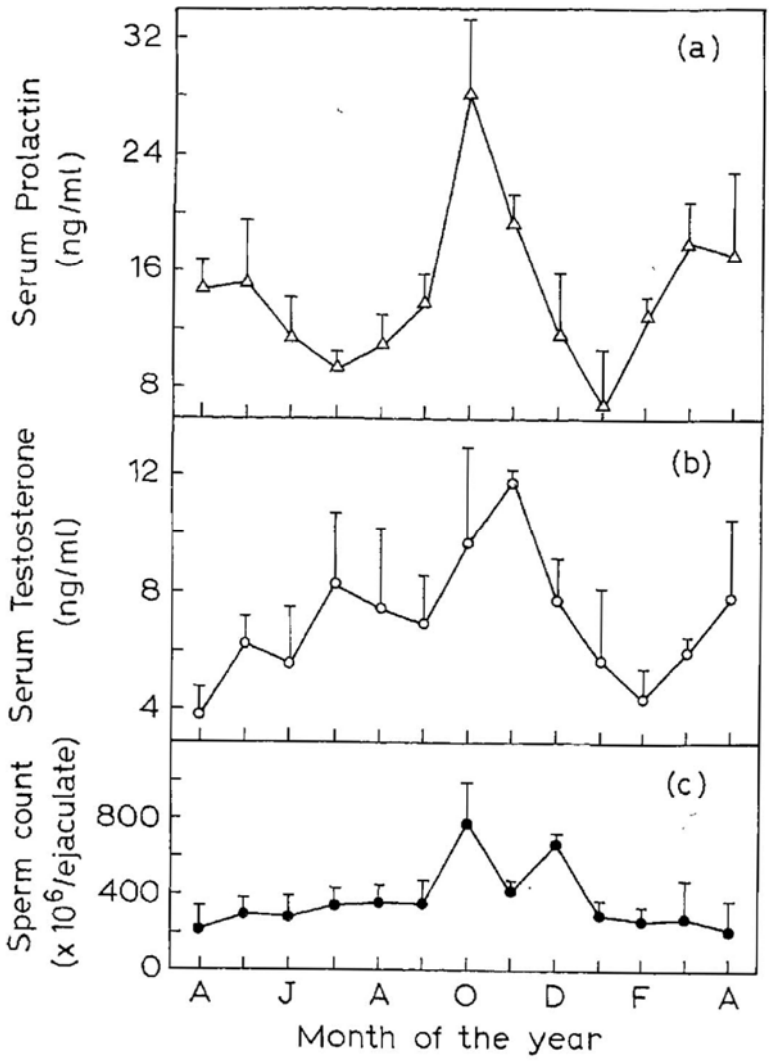

Figure 1. Circulating mean (± SEM) basal serum concentrations of PRL (a) and T (b) in three male bonnet monkeys. Blood was collected between 0800 and 0900 h every month throughout the year. Monthly sperm count (c) observed in bonnet males (n=10) throughout the year.

secretory periods are depicted in figure 4A. While in the month of November (post-monsoon season) the nocturnal serum T reached a peak level of 20·3 ± 1·2 ng/ml and that during March (pre-monsoon) was only 11·0 ±2·1 ng/ml. Furthermore, a significant (P < 0·05) decrease in the level of T secretion during nocturnal hours (1600-1000 h) (calculated as area under curve) was clearly evident during pre/ early-monsoon (mean of March/May/July; AUC 50·5 ± 3·17 sq cm) months compared to that during mid/post-monsoon (mean of September/November/January; AUC-76·33 ± 65·65) months (figure 4B).

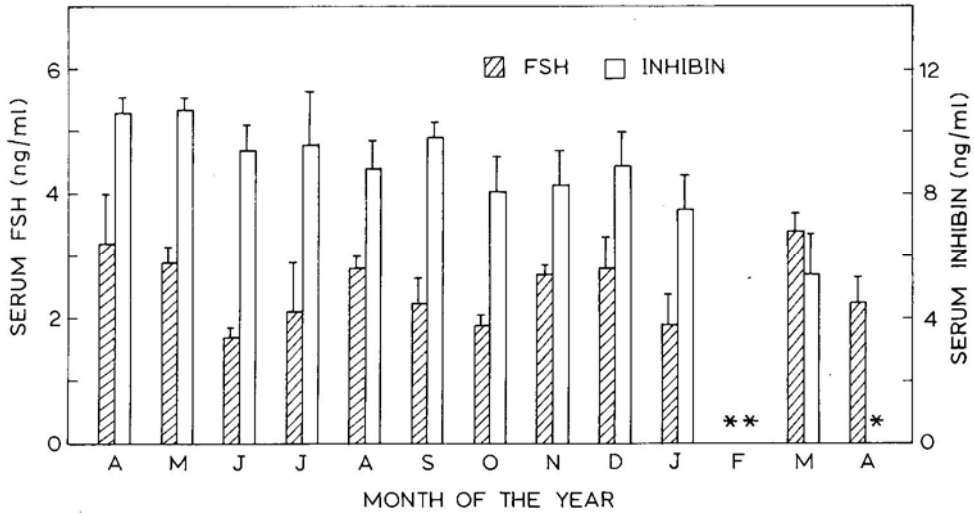

Figure 2. Circulating mean (± SEM) basal concentrations of FSH and INH in three male bonnet monkeys· Blood was collected between 0800 and 0900 h every month through out the year. The asterisks denote that serum was not assayed for the hormones· Mean INH levels remained constant throughout the year except in March.

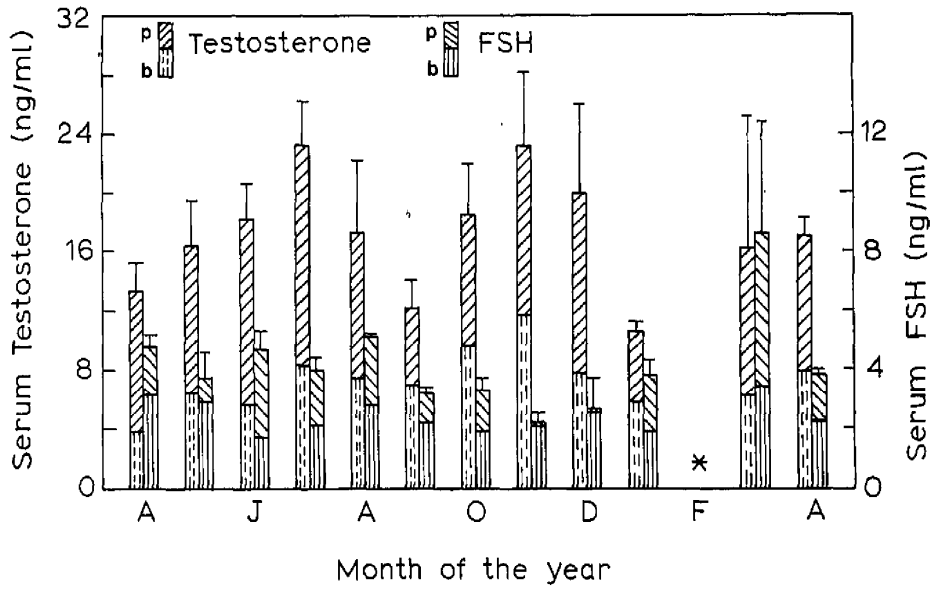

Figure 3. Circulating mean concentrations of T and FSH immediately before and after 10 µg/ml of GnRH injection (iv) in three bonnet monkeys throughout the year.The asterisk denotes that the GnRH test was not carried out in February· Each bar represents mean basal (b) and mean peak (p) hormone secretion· Peak hormone concentrations occurred 40–60 min post GnRH injection.

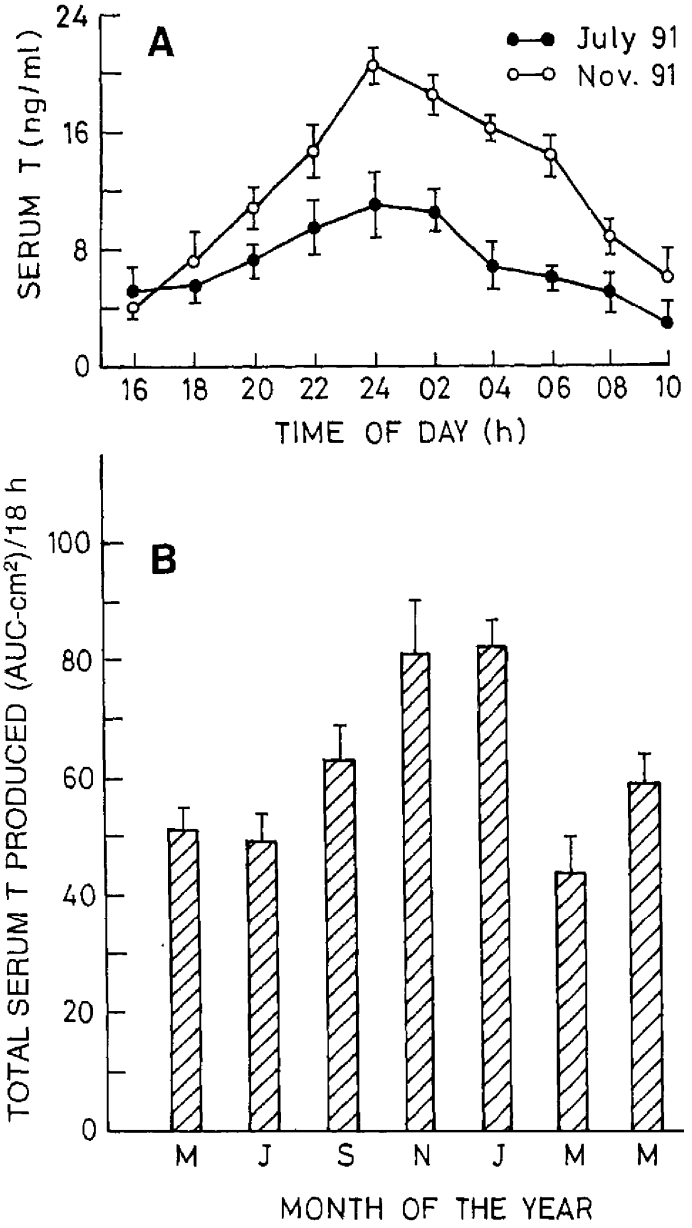

Figure 4. (A) Nocturnal scrum T levels of three male bonnet monkeys (mean ± SEM) in July (●) and November (O) months· **(B)** Total serum T produced in three male bonnet monkeys during alternate months of the year as calculated from area under curve· Values are mean ± SEM.

4. Discussion

The previous observation (Moudgal *et al* 1992) that the adult male bonnet monkey exhibits decreased sperm output and poor quality of spermatozoa during pre and

early-monsoon season appears to be due to an alteration in endocrine hormones. The results of the present study confirm that the secretory pattern of endocrine hormones indeed varies with the time of the year, with circulating T and PRL concentrations being lower during the pre/early-monsoon season. A direct relationship between the endocrine hormone profiles and the sperm output, however, remains to be established in the male bonnet monkey. On the other hand, in the rhesus and Japanese macaque monkeys reproductive performance varies with the season and a direct relationship between the hormone secretory pattern and sexual behaviour has been demonstrated (Michael and Bonsall 1977; Matsubayashi *et al* 1991).In the rhesus male, there is a decline in the sexual activity during February to May months coincident with lower T concentrations (Beck and Wuttke 1979; Conaway and Sade 1965; Michael and Keverne 1971; Plant *et al* 1974). Furthermore, the testicular volume is also lowest during February to May period (Conaway and Sade 1965). In the present study, though the male sexual activity and testicular volume were not monitored, considering that circulating T concentrations were also lower in the bonnet males during February-May months (pre and early-monsoon), it is likely that the testicular volume and sexual activity would be decreased. The significance of annual rhythms in T and PRL concentrations is not clear, but the fact remains that an increased testicular function occurs during September-November months (mid/post-monsoon season)· Since no direct role in the regulation of testicular function has been assigned for pituitary hormones other than gonadotropins, the concomitant occurrence of annual rhythms in T and PRL concentrations may merely reflect the inherently entrained endogenous annual rhythms of these two hormones. However, it should be pointed out that earlier studies from this laboratory have shown that pharmacological alterations of PRL concentrations lead to decreased T concentrations suggesting a common mechanism in the regulation of PRL and T secretion (Rao *et al* 1989).

The finding that exogenous GnRH administration elicited robust increases in serum T concentration suggests that the responsivity of pituitary gonadotropes remains unaltered throughout the year despite changes in basal secretion of this hormone. However, as the dose of GnRH employed in the present study is rather supraphysiological the subtle effects, if any, of the season may not have been discernible with the present experimental protocol· It should be pointed out that although the basal FSH concentrations were unaltered, the nocturnal elevations in T secretion were significantly lower during pre and early-monsoon season compared to the mid and post-monsoon season indicating an overall decline in the functional activity of the hypothalamopituitary-gonadal axis, a finding that further reinforces the view that there may be decreased testicular function and this may account for the reduced sexual activity during pre and early-monsoon season. This decreased activity, as reflected in the lowered reproductive performance, does not appear to be photoperiod related since the monkeys were housed in 12 h L: D cycle controlled rooms throughout the year.

In summary, the results of the present study provide evidence for the occurrence of annual rhythms in T and PRL secretion in male bonnet monkeys similar to the phenomenon previously reported for the rhesus macaque. There appears to be an overall decrease in the functional activity of the hypothalamo-pituitary-gonadal axis during pre and early-monsoon season which may perhaps explain the decreased sperm output observed during this period.

Acknowledgements

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