Profile of organ weights and plasma concentrations of melatonin, estradiol and progesterone during gestation and post-parturition periods in female Indian palm squirrel Funambulus pennanti

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To date, report about the role of pineal gland in maintaining the normal physiology of gestation is scanty. Present study is the first of its kind giving a detail profile of organ weights and plasma concentration of melatonin, estradiol and progesterone to suggest a possible role of pineal gland in maintaining normal physiology during gestation and post-parturition periods of female Indian palm squirrel F. pennanti. Inspite of, inverse pineal-gonadal/melatonin-steroids interrelationship in adult (non-pregnant) females, the present results study suggest a direct relationship of pineal gland activity with ovarian steroids especially during the gestation period. The inverse relationship of melatonin and ovarian steroids is again established after parturition and maintained throughout the life. Thus the pineal gland (activity as judged by its weight, biochemical contents i.e. protein and cholesterol and plasma melatonin level) maintained ovarian/uterine physiology and regulated plasma concentrations of estradiol and progesterone during gestation and post-parturition periods. It is suggested that the pineal gland and its hormone melatonin play an important role to maintain the normal physiology of gestation and the post-partum recovery in Indian palm squirrel F. pennanti.

Until little more than a decade ago, no information was available on whether the pineal gland influences gestation or vice versa and whether pregnancy may affect pineal metabolism and function. But, there are some indications suggesting pineal implications in processes related with gestation and fertility. Rodents exposed to continuous darkness, to short daily photoperiods or to decreased light intensity throughout the gestation period demonstrated significantly shorter pregnancies than controls kept in normal lighting, while long daily photoperiod delayed parturition. However, removal of the pineal gland, which usually mediates between environmental lighting and endocrine glands did not abolish the effect of short photoperiod on the duration of gestation, which suggests that the effect of darkness on gestation may not be mediated by the pineal gland.

Similarly, confusing is the role of pineal gland in fertility processes. While pinealectomy had no effect on the fertility index of rats, binding of hamsters markedly reduced the number of fertile mating and fertility was also reduced in rats subjected to binding and anosmia. These effects could be negated by pinealectomy or chronic melatonin treatment suggesting a progonadal effect of melatonin in pregnant rodents.

To date, not a single report is available explaining the changes occurring in pineal gland during gestation and post-parturition periods related with ovarian/uterine physiology and plasma concentration of estradiol and progesterone. The pineal gland weight, its protein and cholesterol content can be used as markers, therefore, besides the plasma melatonin level they were analysed to judge the pineal gland activity. Further, the gestation period is a very sensitive period of female reproductive cycle and the adrenal physiology reflects directly the stress condition for animals, hence, adrenal gland weight analysis during these periods was also taken into consideration. To throw a light on post-partum recovery process, all these parameters were analysed even after parturition in female Indian palm squirrels.

Materials and Methods

Pregnant female Indian palm squirrels Funambulus pennanti having body weight (120 ± 5g) were collected from the vicinity of Varanasi (Lat. 25°18' N; Long. 83°1' E) during March and April. The pregnancy was initially tested by feeling externally the bulging of uterus with implanted embryo and

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finally with Preg-test (Intercare Pharmaceuticals, Calcutta). All the pregnant females were kept in separate wire net cages (25"x25"x30" in size) individually in an animal room fully exposed to ambient condition of light, temperature and humidity. They were provided with water soaked gram (Cicer arietinum) and water ad libitum.

For determination of total gestation period, the days starting from initial implantation till date of parturition was found to be -45±2. With extensive observation and experiencing the size of implanted embryo intoto (by feeling externally), the pregnant females were divided into 11 groups i.e. GP3, GP6, GP9, GP12, GP15, GP20, GP25, GP30, GP35, GP40, GP45 (GP: gestation period followed by numerical representing the days: Table I; Figs 1 and 2). Three groups of delivered females i.e. AP5, AP10, AP20 (AP: after parturition followed by numerical representing the days: Fig 3) were taken into consideration to note post-parturition condition and to define the phenomenon of post-partum recovery. One group of non-pregnant (NP) females was used to note comparative changes occurring in organ weights and plasma concentrations of melatonin, estradiol and progesterone during gestation and post-parturition periods. Different groups of pregnant females were sacrificed by decapitation at night (2200 – 2300 hrs). The maternal pineal, ovary, uterus and adrenal gland were dissected out and weighed.

For biochemical analyses, the pineal gland after washing in normal saline (0.9% NaCl) homogenised in a micro glass homogenizer. Pineal protein content was estimated spectrophotometrically by Folin-Ciocalteau reagent7, while the cholesterol content with the help of Liebermann-Burchard reagent8.

For hormonal analyses, the plasma melatonin content of respective animals was estimated following the radioimmunoassay method9,10. The plasma concentrations of estradiol and progesterone were estimated by commercial radioimmunoassay kits; Estradiol (125I) coated RIA kit from Orion Diagnostics, Espoo, Finland and progesterone (125I) radioimmunoassay kit from Binax, Portland, MAINE, USA. The validation data of above hormonal assays are given in Table 2.

Results

In comparison to non-pregnant (NP) females the body weight of pregnant females increased gradually during the gestation period and then decreased after parturition (Fig. 4). No change in adrenal gland weight was noted during gestation and post-parturition periods (Fig. 4). Following a peak value of ovarian weight at initial implantation of embryo, it decreased gradually with advancement of pregnancy and after parturition again exhibited a gradual increase (Fig. 5). In comparison to NP females, uterine weight of pregnant females increased gradually with fetal development till parturition and then decreased sharply after parturition (Fig. 5). The plasma levels of estradiol and progesterone in pregnant females increased gradually during gestation period. After parturition, plasma level of progesterone decreased sharply while estradiol decreased gradually (Fig. 6). In comparison to NP females the pineal gland weight and plasma melatonin level of pregnant females attained a lowest value at initial implantation of embryo and increased gradually during gestation period and even after parturition (Fig. 7). Similarly, the pineal protein content after attaining a lowest value at initial implantation of embryo exhibited

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<th>Table 1—Size of embryo (in toto) at different gestation periods</th>
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Fig. 1—Size of embryo (in toto): NP: non-pregnant, GP: gestation period and numericals representing the days.

Fig. 2—In utero age of fetus (GP: gestation period and numericals representing the days).

Fig. 3—Post-partum uterus (AP: after parturition period and numericals representing the days).
increasing trend during pregnancy and even after parturition, however, no change in pineal cholesterol content was noted during gestation and post-parturition periods (Fig. 8).

Discussion
The reproductive active phase in tropics for seasonally breeding rodent is marked with availability of food and favourable environmental conditions

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**Fig. 4**—Changes in body and adrenal gland weight during gestation and after parturition (NP: non-pregnant; GP: gestation period followed by days; AP: after parturition followed by days: One way ANOVA: for body weight, $P<0.001$ and for adrenal gland weight, $P>0.2$).

**Fig. 5**—Changes in ovarian and uterine weight during gestation period and after parturition (NP: non-pregnant; GP: gestation period followed by days; AP: after parturition followed by days: One way ANOVA: Ovarian weight, $P<0.001$ and Uterine weight, $P<0.001$).
(moderate temperature and humidity) with sufficient place for their movement and mating. These factors are available for Indian subtropics during February to April. Hence, maximum mating of Indian palm squirrels occurred during February and March. A large number of pregnant females could be noted during March and April in nature. Quite less number of pregnant females were also during August and September, when the above eco-socio factors were moderately available in a tropical country. After

![Graph 1](image1)

**Fig. 6—**Changes in plasma levels of estradiol and progesterone during gestation period and after parturition (NP: non-pregnant; GP: gestation period followed by days; AP: after parturition followed by days: One way ANOVA, \( P \) for estradiol and progesterone <0.001).

![Graph 2](image2)

**Fig. 7—**Changes in pineal gland weight and plasma level of melatonin during gestation period and after parturition (NP: non-pregnant; GP: gestation period followed by days; AP: after parturition followed by days: One way ANOVA, \( P \) for pineal gland weight and plasma melatonin <0.001).
successful mating, fertilization of ovum with sperm occurs and finally the uterine wall is being implanted with embryos (number of implanted embryo: 1-5 in case of Indian palm squirrel). During gestation period, gradual thickening of uterine wall (to maintain the developing embryos) and accumulation of fat in lower abdominal area (to fulfil the demand of lactation) increases the female's body weight. After parturition, uterus again starts to shrink to maintain its normal physiology and finally to make itself suitable for next implantation of embryos. Hence, the uterine weight decreased gradually after parturition in these squirrels. The accumulated fat in the lower abdominal area gets metabolized to fulfil the energetic demand of lactation, hence female's body weight decreased gradually after parturition as well. An sudden decrease in female's body weight was also registered just after parturition due to delivery of pups, which shared maximally their weight with female's body during pregnancy. The loss of body weight in female squirrels in weaning and feeding of young ones favoured the idea of Whittier and Crews\(^1\) suggesting that females bear the maximum cost of reproduction in all vertebrates.

A peak activity of ovarian physiology was required for successful mating and possible implantation of embryos, hence a maximum ovarian weight was noted at the beginning of gestation period. Following implantation of embryo, ovarian weight decreased gradually till parturition and after parturition, it increased gradually till next breeding (reproductive active phase) to attain normal ovarian physiology. The ovarian steroidogenesis i.e. synthesis and release of estradiol and progesterone marked by their plasma concentrations in squirrels increased gradually during gestation period and attained highest value at parturition. After parturition, the plasma progesterone level declined sharply, as it was required to maintain the pregnancy only. However, the plasma estradiol level declined gradually as it was required to maintain the normal physiology of gestation as well as to facilitate lactation in delivered females. The maintenance of uterine physiology is a progesterone dependent phenomenon, hence a higher plasma level of progesterone and increased uterine weight was also required to protect and maintain the implanted embryos.

Several authors todate reported the inverse relationship of pineal gland activity with ovarian weight and plasma levels of ovarian steroids in normal female rodents\(^2,13\). Pineal gland activity was inversely correlated with reproductive activity of
Indian palm squirrel\textsuperscript{14}. The present study for the first time suggested a direct relationship of pineal gland activity with plasma levels of estradiol and progesterone especially during the gestation period. The pineal gland activity as judged by its weight, protein content and plasma melatonin level was lowest at the time of implantation of embryos during the reproductive active phase of Indian palm squirrel. Following implantation, along with gradual increase in plasma levels of estradiol and progesterone, gradual increase in pineal gland activity was registered throughout the gestation period (from initial implantation to date of parturition). Therefore, it may be suggested that high level of plasma melatonin and ovarian steroids (a direct relationship) is required for the maintenance of normal physiology of gestation. Interestingly, after parturition, the inverse relationship of melatonin and ovarian steroids is again established (i.e. high level of plasma melatonin with low level of plasma estradiol and progesterone) and maintained throughout the life. The exogenous melatonin treatments\textsuperscript{14} and photoperiodic alterations\textsuperscript{15} leads to change in pineal gland activity before 30\textsuperscript{th} day of gestation resulting in resorption of implanted embryo\textsuperscript{15}, while after 30\textsuperscript{th} day of gestation it did not interfere with the maintenance of pregnancy but altered the neonatal growth and sexual development\textsuperscript{16}. The change in pineal gland activity following exogenous melatonin treatment\textsuperscript{14} and different photoperiodic exposures\textsuperscript{15} disturbs the hormonal profiles of estradiol and progesterone during early gestation period\textsuperscript{14,15} and finally results in major changes of female’s ability to maintain ovarian/uterine function, thereby causing resorption of implanted embryos. Later in pregnancy, the placenta may support the ovary/uterus to some extent, so that the effects of the changes in pineal gland activity on plasma levels of estradiol and progesterone are inconsequential, hence female delivers pups normally but with altered growth and sexual maturation\textsuperscript{15}. It is now well established that the receptors of gonadal steroids (especially estradiol and testosterone) were present in the pineal\textsuperscript{17}, whereas the melatonin receptors were present in the gonads\textsuperscript{18}. Hence it may be possible that the gonadal steroids and pineal melatonin regulates the plasma levels of each other. Further, the receptors of melatonin in pars-tuberinalis show seasonal variation following which the steroid hormone level changes in plasma\textsuperscript{19}. Surprisingly, when the plasma estradiol level increased during gestation, it also enhanced the pineal gland activity and facilitated the synthesis and release of melatonin rather than inhibiting it as it does in normal female rodents\textsuperscript{12,13}.

Therefore, it may be concluded that despite of melatonin-steroids inverse relationship in adult (non-pregnant) females, a direct relationship is maintained between plasma levels of melatonin and ovarian steroid especially during pregnancy (starting from date of implantation till date of parturition) for this rodent. After parturition, the inverse melatonin-steroid relationship is again established to enable these rodents to recover themselves for consequential reproductive cycle (post-partum recovery). Thus, the pineal gland and its hormone melatonin play an important role to maintain the normal physiology of gestation.

The reproductive activity of this rodent needs further an elaboration as it shows a typical post-partum recovery. On the basis of present study we may divide this post-partum recovery phase of female into 6 important physiological steps, which take about 2 months time in total. It begins with (a) gradual increase in plasma progesterone level following its sharp decline during parturition followed by, (b) gradual decrease in uterine weight, (c) an increase in ovarian weight after a week or two, (d) gradual decrease in body weight due to metabolism of fat in lower abdominal area, (e) an increase in plasma estradiol level following its gradual decrease after parturition, and (f) an increase in pineal gland activity (i.e. pineal gland weight, pineal protein content and plasma level of melatonin).

**Acknowledgement**

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