Pattern of Plasma Sex Steroid Hormone Levels during Reproductive Cycles of Male and Female Tropical Lizard, Calotes versicolor

R. S. Radder, B. A. Shanbhag,1 and S. K. Saidapur
Department of Zoology, Karnatak University, Dharwad 580 003, India

Accepted August 10, 2001

Annual changes in gonadal activity and plasma sex steroid hormone levels in male and female Calotes versicolor [plasma testosterone (T) levels in males and 17 β-estradiol (E2) and progesterone (P) levels in females] are described. In females both plasma E2 (32.80 ± 12.91 pg/ml) and P (1.72 ± 0.79 ng/ml) levels are at their low levels during the postbreeding season when the gonads are regressed. With the onset of recruitment of vitellogenic follicles, E2 levels begin to rise, reaching peak values (1306.00 ± 407.01 pg/ml) when the follicles are preovulatory. During this time, plasma levels of P are low. Plasma E2 levels decline (285.60 ± 143.0 pg/ml) soon after ovulation, and circulating concentrations of P begin to rise, reaching peak value (19.24 ± 10.03 ng/ml), during eggshell formation. In gravid females, at mid-gestation, the same pattern of E2 secretion is found when a second set of follicles undergoes vitellogenesis. However, P levels remain low from mid-gestation (4.06 ± 2.17 ng/ml) until oviposition. These findings suggest that high P levels are not needed for oviductal egg retention during late gestation and that low levels of P may facilitate growth of a new batch of vitellogenic follicles. In males, plasma levels of T are correlated with the spermatogenic activity that accompanies breeding.

Key Words: Calotes versicolor; estradiol; multiclutch lizard; progesterone; reproductive cycle; testosterone.

INTRODUCTION

Seasonal changes in the reproductive events and plasma steroid levels are documented in several species of lizards (Arslan et al., 1978a, b; Courty and Dufaure, 1982; McKinney and Marion, 1985; Ando et al., 1990, 1992; Diaz et al., 1994; Jones et al., 1997; Tokarz et al., 1998; Phillips and Millar, 1998; Amey and Whittier, 2000). These studies show that seasonal gonadal recrudescence and elevation in plasma sex steroids are correlated events. Also, seasonal activation in sexual and territorial behaviors generally coincides with elevation of plasma sex steroids. Such studies are limited mostly to species with one reproductive event per breeding season, with the exception of that on Pogona barbata (Amey and Whittier, 2000), which is a multiclutch lizard. In a given population of P. barbata, therefore, individuals in various reproductive states are found during the breeding season. However, it is not clear whether reproductive events such as vitellogenic growth of follicles and gravidity overlap or not in an individual lizard. Studies on species with overlapping reproductive events within an individual are lacking. However, studies on such species are needed to provide critical information with regard to the role of sex steroids in the maintenance of gravidity and the simultaneous sustaining of the growth of vitellogenic follicles.

The lizard Calotes versicolor is widely distributed in India and is a seasonal breeder (Gouder and Nad-
Males are spermatogenetically active during April–September (Gouder and Nadkarni, 1979). Gravid lizards are encountered during May–October (Shanbhag and Prasad, 1993; Shanbhag et al., 2000a). Females are reproductively inactive from December to April. They are polyautochronic and multiclutched (Shanbhag et al., 2000a). Consequently, during a breeding phase, gravid females with vitellogenic follicles are also encountered. Hence, C. versicolor forms a good model for the study of the pattern of steroid cycles as it exhibits simultaneously maintenance of gravidity (probably a progesterone-dependent event) and growth of vitellogenic follicles (an estrogen-dependent event). The present study was therefore undertaken to determine the pattern of changes in plasma 17β-estradiol (E₂) and progesterone (P) profiles in females and that of testosterone (T) profiles in males of C. versicolor during different phases of the reproductive cycle and to correlate these with the gonadal condition.

**MATERIALS AND METHODS**

**Animals**

Adult female C. versicolor [snout–vent length (SVL) > 8.5 cm] were collected from areas surrounding Dharwad city (15°17’N and 75°3’E) at bimonthly intervals from December 1997 to April 1998 and at monthly intervals from May to October 1998. Adult males were sampled at bimonthly intervals from December 1997 to October 1998. All specimens were collected during the first week of the respective months. The lizards of each sex (5–10) were autopsied 1 day after they were brought to the laboratory. At autopsy, SVL (cm) and mass (g) of the body, gonads, oviducts, and fat bodies were recorded. The gonads were fixed in Bouin’s fluid and processed for histological studies.

**Categorization of Individuals Based on Reproductive Status**

**Females.** In C. versicolor ovaries, similar-sized follicles are organized into distinct groups. The total number and diameter of follicles belonging to a group having the largest-sized follicles were recorded from the left ovary. The follicles <1 mm diameter were measured with an ocular micrometer and those >1 mm diameter were measured with a micrometer. Follicles >2.5 mm diameter represent vitellogenic status (Shanbhag and Prasad, 1993).

Data for females, for quiescent and recrudescent phases (December and April), were considered on the basis of the bimonthly sample collection. Since ovarian follicular development, ovulation, oviposition, etc., occur asynchronously among the members in a population, individuals in different reproductive states are encountered between May and October. Therefore, the data from the breeding phase were classified with respect to the reproductive status of the individual females rather than on a monthly basis as follows: (1) early vitellogenic stage, initiation of vitellogenesis in the ovary (ovary with vitellogenic follicles of diameter 2.5–5.0 mm); (2) preovulatory stage, with vitellogenic follicles (5.1–8.0 mm diameter), without oviductal eggs; (3) postovulatory stage, eggs in the oviducts but shell not formed; (4) early gestation stage, shelled eggs with embryos at primitive streak, no vitellogenic follicles; (5) mid-gestation stage, embryos at stages 14–20, with a new batch of vitellogenic follicles (diameter 2.5–5.0 mm); and (6) late-gestation or preoviposition stage, embryos between stages 25 and 27 with large vitellogenic follicles of diameter 5.1–8.0 mm. The lizards devoid of vitellogenic follicles in this stage were considered separately for the observation.

**Males.** The testes were cut at 5 μm thickness and stained with hematoxylin–eosin. The diameters of the testis, seminiferous tubule, and Leydig cell nucleus were measured with an ocular micrometer as described previously (Sharma and Shanbhag, 1992). The monthly means were then calculated. Based on the presence and density of different stages of spermatogenesis (subjective gradation), the testicular activity was categorized into six stages: stage I, presence of spermatogonia only; stage II, presence of spermatogonia and primary and secondary spermatocytes; stage III, presence of spermatogonia and spermatocytes, moderate number of spermatids; stage IV, presence of spermatogonia and spermatocytes, a greater number of spermatids and sperm; stage V, presence of residual spermatids and sperm in the lumen; and stage VI, seminiferous tubules lined by a single layer of spermatogonia and Sertoli cells.
Enzyme-Linked Immunosorbent Assay for Plasma Steroid Hormones

The serum samples were obtained from each lizard as described previously by Shanbhag et al. (2000b). Plasma T in the males and E₂ and P in the females were measured according to protocols supplied with the respective kits (Sentinel CH, Italy).

**Plasma E₂ and P.** Sensitivity for E₂ was 1 pg according to the manufacturer’s protocol and allowed the determination of E₂ from 20 to 4000 pg/ml. The recoveries of 20, 120, 800, and 4000 pg/ml E₂ added to buffer solution were 85.52, 90.3, 95.62, and 98.44%, respectively, with respect to original concentrations. Precision of intra- and interrun assays had coefficients of variation of 4.82 and 6.78%, respectively.

Sensitivity for the P assay was 2 pg with reference to manufacturer’s protocol. This method allowed the determination of P from 0.4 to 20.0 ng/ml. The recovery rates were 108, 115, 107, and 104% with reference to 0.4, 1.2, 3.0, and 8.0 ng/ml of P added to buffer solution, respectively. Precision for intra- and interrun assays had coefficients of variation of 3.28 and 5.64%, respectively.

**Plasma T.** Sensitivity of the method was 5 pg according to manufacturer’s protocol. The method allowed the determination of T from 0.2 to 40.0 ng/ml. Accuracy of the assay was determined by addition of 0.2, 1.0, 3.0, and 8.0 ng/ml of T to saline solution. Recovery rates for 0.2, 1.0, 3.0, and 8.0 ng/ml T were 115, 130, 110, and 102.5%, respectively. Precision of intra- and interrun assays had coefficients of variation of 4.2 and 7.3%, respectively.

Statistical Analysis

Mean ± SE for all the recorded traits was computed from untransformed data. Carl Pearson correlation and multiple regression analysis were used to assess the relationships among body condition, residual gonadal mass, oviduct mass, fat body mass, and circulating levels of T, E₂, and P. Body condition was obtained by regression of body mass on SVL. Residuals for gonadal, fat body, and oviduct masses and for diameter of testis, seminiferous tubule, and Leydig cell nucleus were generated by regression of each trait on SVL. For females, variation in the reproductive traits and steroid hormone levels were analyzed separately for individuals of the nonbreeding period (December to April), and individuals in different reproductive states (the breeding period) were analyzed by analysis of variance (ANOVA) followed by Tukey–Honestly significant difference (Tukey’s–HSD). Variations in the above parameters and plasma steroid hormone levels in male lizards were also analyzed by ANOVA followed by the Tukey’s–HSD multiple range test with months as a grouping factor. Before generation of residuals and ANOVA, all data were log transformed to meet the assumptions of parametric tests. The probability level was set at 0.05 for all the tests. SPSS (version 6.1.3 for Windows) software was used for analysis.

RESULTS

**Plasma E₂ and P Titers and Ovarian Cycle**

The pattern of variations in the circulating levels of plasma E₂ and P during the nonbreeding (December–April) and breeding (May–October) phases in relation to the growth of vitellogenic follicles is graphically represented in Fig. 1. Plasma E₂ levels increased commensurate with growth of ovarian follicles ($r = 0.96$, $P < 0.01$) vis-à-vis ovarian mass ($r = 0.94$, $P < 0.01$). Plasma P rose to peak level following ovulation, during early gestation stage with oviductal eggs. Plasma E₂ levels and oviduct weight are positively correlated ($r = 0.68$, $P < 0.01$), whereas plasma E₂ levels are negatively correlated with fat body mass ($r = -0.62$, $P < 0.01$). An inverse relationship between plasma E₂ and P was apparent during the breeding phase ($r = -0.63$, $P < 0.01$). During the postbreeding and the recrudescence phases, levels of both plasma E₂ and P were minimal.

Between December and February, the ovaries and oviducts were in the regressed state (Table 1). The largest follicles measured $\approx 0.5$ mm in diameter (Table 1) and plasma E₂ and P were at basal levels (Fig. 1). By April, the ovarian and oviductal masses increased compared to those in February (Table 1). The ovaries contained follicles of $\approx 1.5$ mm diameter (Table 1). Plasma E₂ levels were elevated ($110.60 \pm 29.64$ pg/ml; Fig. 1) but those of plasma P remained unchanged ($1.74 \pm 0.93$ ng/ml; Fig. 1) compared to those in previous months.
During the breeding phase (May–October) plasma E2 and P concentrations showed significant variations among the individual lizards depending upon the reproductive stages (ANOVA, df 5, 27, F 5 34.45, P < 0.0001, and df 5, 27, F 5 9.26, P < 0.001). With the initiation of vitellogenesis, increases in the masses of ovary and oviduct and in the diameter of the largest follicles were observed (Table 1). Subsequently, an increase in plasma E2 (647.33 ± 28.99 pg/ml) was also evident during this stage. However, there was no notable variation in the plasma P levels (Fig. 1). In preovulatory individuals, plasma E2 levels were highest compared to the other stages (1306.00 ± 407.01 pg/ml; Fig. 1). In such individuals the ovaries and oviduct weighed significantly higher (Table 1) and the plasma P concentrations were significantly lower (1.84 ± 0.72 ng/ml, Fig. 1) than in other stages of breeding. In the postovulatory stage, i.e., a few hours following ovulation, there were significant drops in plasma E2 levels (285.60 ± 143.00 pg/ml; Fig. 1) and in ovarian mass compared to that of the preovulatory stage (Table 1). Following ovulation there was a steady increase in the plasma P titers (5.33 ± 3.26 ng/ml; Fig. 1).

During the early gestation stage, plasma E2 levels further dropped and reached the lowest values (91.20 ± 19.12 pg/ml; Fig. 1). The ovaries contained previtellogenic follicles of about 2 mm diameter and their masses were significantly reduced (Table 1). Plasma P reached peak values during this stage (19.24 ± 10.03 ng/ml; Fig. 1), coinciding with eggshell formation. By the mid-gestation stage, ovarian mass

![FIG. 1. Changes in plasma E2 and P levels (mean ± SE) during nonbreeding and breeding phases in female Calotes versicolor. EV, early vitellogenic; V, vitellogenic; PO, postovulatory; EG, early gestation; MG, mid-gestation; LG, late gestation or preoviposition stage during breeding phase. Postbree., postbreeding; Recr., recrudescence phase.]

| TABLE 1 |
| Changes (Mean ± SE from Untransformed Data) in Body, Ovary, Oviduct, and Fat Body Masses and in Diameter of Largest Follicles in Calotes versicolor during a Reproductive Cycle (n = Sample Size) |

<table>
<thead>
<tr>
<th>Month</th>
<th>n</th>
<th>SVL (cm)</th>
<th>Body (g)</th>
<th>Ovary (g)</th>
<th>Oviduct (g)</th>
<th>Fat body (g)</th>
<th>Diameter of largest follicle (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>5</td>
<td>10.20 ± 0.22</td>
<td>45.60 ± 2.56</td>
<td>0.06 ± 0.02</td>
<td>0.28 ± 0.06*</td>
<td>1.32 ± 0.08*</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>February</td>
<td>5</td>
<td>10.00 ± 0.48</td>
<td>35.40 ± 10.16</td>
<td>0.03 ± 0.01</td>
<td>0.11 ± 0.08*</td>
<td>1.03 ± 0.71*</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>April</td>
<td>5</td>
<td>10.28 ± 0.64</td>
<td>49.80 ± 7.40</td>
<td>0.11 ± 0.02</td>
<td>0.42 ± 0.10</td>
<td>1.80 ± 0.24*</td>
<td>1.47 ± 0.16</td>
</tr>
<tr>
<td>Breeding phase (May–October)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early vitellogenic</td>
<td>3</td>
<td>10.20 ± 0.15</td>
<td>44.67 ± 1.76</td>
<td>1.12 ± 0.44bc</td>
<td>0.51 ± 0.10</td>
<td>0.76 ± 0.11</td>
<td>3.77 ± 0.21bc</td>
</tr>
<tr>
<td>Preovulatory</td>
<td>5</td>
<td>10.44 ± 0.24</td>
<td>47.80 ± 7.43</td>
<td>2.84 ± 1.29abcd</td>
<td>0.57 ± 0.09</td>
<td>0.01 ± 0.01</td>
<td>6.30 ± 1.08abcd</td>
</tr>
<tr>
<td>Postovulatory</td>
<td>5</td>
<td>10.54 ± 0.42</td>
<td>49.80 ± 12.45</td>
<td>0.28 ± 0.11</td>
<td>0.53 ± 0.13</td>
<td>0.05 ± 0.05</td>
<td>1.96 ± 0.30</td>
</tr>
<tr>
<td>Early gestation</td>
<td>5</td>
<td>10.00 ± 0.65</td>
<td>40.60 ± 8.47</td>
<td>0.22 ± 0.16</td>
<td>0.45 ± 0.15</td>
<td>0.02 ± 0.02</td>
<td>2.22 ± 0.16</td>
</tr>
<tr>
<td>Mid-gestation</td>
<td>5</td>
<td>10.38 ± 0.61</td>
<td>47.00 ± 9.38</td>
<td>0.86 ± 0.18bc</td>
<td>0.48 ± 0.14</td>
<td>—</td>
<td>3.55 ± 0.51bc</td>
</tr>
<tr>
<td>Preoviposition</td>
<td>5</td>
<td>10.44 ± 0.24</td>
<td>47.20 ± 4.21</td>
<td>1.80 ± 0.24bc</td>
<td>0.58 ± 0.03</td>
<td>—</td>
<td>5.41 ± 0.51abcd</td>
</tr>
</tbody>
</table>

**Note.** Superscripts a, b, c, and d indicate significant differences from early vitellogenic, postovulatory, and early and mid-gestation stages, respectively.

*Significantly different from breeding phase.
showed an increasing trend due to the appearance of the second set of vitellogenic follicles of 2.5 to 5.0 mm diameter (Table 1). Subsequently, in these lizards plasma E₂ levels increased (647.00 ± 222.02 pg/ml; Fig. 1) compared to those in the previous stage. However, plasma P titers began declining in this stage (4.34 ± 2.77 ng/ml). Lizards in the late gestation stage exhibited further increases in the ovarian mass, size of the largest follicles (Table 1), and plasma E₂ concentrations (1040.00 ± 270.19 pg/ml; Fig. 1). However, lizards devoid of vitellogenic follicles (n = 4) in the ovary during this stage had low plasma E₂ levels (131.50 ± 21.91 pg/ml). Plasma P levels remained low until oviposition (4.06 ± 2.17 ng/ml).

**Plasma T Titers and Testis Cycle**

Bimonthly variations in plasma T concentrations and testis masses are graphically represented in Fig. 2A. Plasma T levels differed significantly among different months (ANOVA, df 5, 27, F = 9.92, P < 0.0001). T levels varied significantly with testicular mass as revealed by multiple regression analysis with SVL and body conditions as covariates (r² = 0.29, F = 6.23, P < 0.01). Changes in plasma T levels were correlated with body condition (r = 0.54, P < 0.01) and residual testis mass (r = 0.77, P < 0.01). However, plasma T levels and fat body mass were inversely correlated (r = −0.41, P < 0.05).

In December, the testes weighed least and showed stage I of testicular activity (Table 2). The testis mass, the diameters of the testis, seminiferous tubule, and Leydig cell nucleus, and the plasma T levels (mean 0.55 ± 0.03 ng/ml; Fig. 2A) were minimal (Table 2). In February, there was an increase in the size of testis, seminiferous tubule, and Leydig cell nucleus (Table 2). Stage II testicular activity was seen in these lizards. The plasma T levels were slightly elevated over the December values (0.86 ± 0.18 ng/ml; Fig. 2A).

In April, the testes were fairly well developed with a further increase in their mass and in the diameters of testis, seminiferous tubule, and Leydig cell nucleus (Table 2). The lizards showed stage III testicular activity. Plasma T titers increased significantly compared to that in February (6.15 ± 1.53 ng/ml; Fig. 2A). In June, the testis mass and the diameters of testis, seminiferous tubules, and Leydig cell nucleus reached peak values (Table 2). Plasma T levels rose to a mean of 12.92 ± 1.19 ng/ml (Fig. 2A). In all the lizards stage IV testicular activity was seen.

In August, the testis mass and diameter began declining (Table 2) but plasma T levels still remained elevated (10.66 ± 0.66 ng/ml; Fig. 2A). Of the five lizards three showed stage IV and two stage V testicular activity. By October, testicular regression was apparent in all lizards. The testis mass and the diameters of testis, seminiferous tubules, and Leydig cell nucleus were significantly lower than those in the previous month (Table 2). Seminiferous tubules were lined by a layer of spermatogonia and Sertoli cells only (stage VI). Plasma T declined significantly compared to the values of April–August (2.02 ± 1.22), the breeding phase.
Figure 2B depicts the relationship between plasma T titers and testicular activity. At the beginning of testicular recrudescence (stages I and II of testicular activity) plasma T levels were low. But the T levels increased during stage III. The plasma levels of T were highest in individuals with stage IV testicular activity. Plasma T levels declined moderately following the onset of testicular regression (stage V) but declined sharply thereafter with advancement in testicular regression (stage VI).

DISCUSSION

The pattern of changes in plasma $E_2$ seen in $C.\ versicolor$ in general corroborates the findings on other lizard species (Jones et al., 1983, 1997; Carnevali et al., 1991). In $C.\ versicolor$, $E_2$ levels are low in nonreproductive females with small previtellogenic follicles and those in early gestation. Estradiol levels increase in vitellogenic females, and highest levels of the hormones are associated with the presence of large vitellogenic follicles. The oviduct growth is maximum when high $E_2$ levels are found. In contrast, in $P.\ barbata$, $E_2$ was detectable in very low quantities in a few lizards irrespective of ovarian reproductive state (Amey and Whittier, 2000).

In most of the lizard species studied so far the vitellogenic and gravid stages are temporally separated. However, in $C.\ versicolor$, both these phases overlap in individuals at certain times. In gravid lizards with vitellogenic follicles, an inverse relationship between plasma $E_2$ and P is observed. Also, the pattern of plasma $E_2$ and P levels among gravid females signifies a specific role for each of the hormones. The gravid lizards in early gestation exhibit low $E_2$ and high P levels. These lizards do not contain vitellogenic follicles, the major source for plasma $E_2$. The highest P levels in these lizards coincide with the eggshell production, suggesting its role in the formation of the shell as reported in other species of lizards (Arslan et al., 1978b; van Wyk, 1984; Moore et al., 1985; Masson and Guillette, 1987; Fox and Guillette, 1987; Diaz et al., 1994).

Gravid $C.\ versicolor$ in mid-gestation possess ovaries with the vitellogenic follicles of the subsequent clutch and exhibit a drop in P and a rise in $E_2$. A decline in P levels in gravid lizards in mid-gestation with vitellogenic follicles seems to facilitate recruitment and growth of the subsequent set of vitellogenic follicles. In fact in gravid $C.\ versicolor$, plasma P levels steadily decline after the formation of eggshell as in other gravid individuals not possessing vitellogenic follicles of the subsequent clutch. Similarly, in most lizard species plasma P levels decline after mid-gestation (Arslan et al., 1978b; van Wyk, 1984; Moore et al., 1985; Masson and Guillette, 1987; Fox and Guillette, 1987; Diaz et al., 1994) except in $Sceloporus\ jarrovi$ (Guillette et al., 1981). The present study suggests that in $C.\ versicolor$, both these phases overlap in individuals at certain times.

### Table 2

<table>
<thead>
<tr>
<th>Month</th>
<th>n</th>
<th>SVL (cm)</th>
<th>Body</th>
<th>Testis</th>
<th>Fat body</th>
<th>Testis (mm)</th>
<th>Seminiferous tubule (μm)</th>
<th>Leydig cell nucleus (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>7</td>
<td>10.60 ± 0.33</td>
<td>46.57 ± 3.63</td>
<td>0.04 ± 0.01</td>
<td>0.90 ± 0.36</td>
<td>1.39 ± 0.09</td>
<td>69.18 ± 2.54</td>
<td>2.30 ± 0.06</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>5</td>
<td>11.02 ± 0.90</td>
<td>48.40 ± 7.64</td>
<td>0.06 ± 0.01</td>
<td>0.71 ± 0.34</td>
<td>1.87 ± 0.14</td>
<td>91.34 ± 4.84</td>
<td>2.77 ± 0.21</td>
</tr>
<tr>
<td>April</td>
<td>5</td>
<td>11.48 ± 0.35</td>
<td>54.80 ± 2.47</td>
<td>0.72 ± 0.07</td>
<td>3.37 ± 0.16</td>
<td>4.33 ± 0.19</td>
<td>328.72 ± 16.95</td>
<td>5.62 ± 0.13</td>
</tr>
<tr>
<td>June</td>
<td>5</td>
<td>10.28 ± 0.25</td>
<td>45.40 ± 2.35</td>
<td>1.13 ± 0.12</td>
<td>0.03 ± 0.03</td>
<td>5.23 ± 0.26</td>
<td>329.14 ± 14.83</td>
<td>6.20 ± 0.14</td>
</tr>
<tr>
<td>August</td>
<td>5</td>
<td>11.22 ± 0.25</td>
<td>54.60 ± 2.71</td>
<td>1.06 ± 0.13</td>
<td>0.01 ± 0.01</td>
<td>4.86 ± 0.34</td>
<td>271.44 ± 19.04</td>
<td>5.56 ± 0.21</td>
</tr>
<tr>
<td>October</td>
<td>5</td>
<td>11.15 ± 0.33</td>
<td>51.83 ± 2.91</td>
<td>0.06 ± 0.01</td>
<td>0.47 ± 0.20</td>
<td>2.10 ± 0.10</td>
<td>95.40 ± 1.33</td>
<td>2.32 ± 0.06</td>
</tr>
</tbody>
</table>

Note. Superscripts a (December), b (February), c (April), d (June), e (August), and f (October) indicate significant differences from respective months at $P < 0.05$. Analyses are based on residuals of each trait.
**versicolor** high levels of P may not be needed during oviductal egg retention in later stages of gestation. Earlier studies on lizards involving lutectomy have also suggested that P is not needed for egg retention beyond mid-gestation (Roth et al., 1973; Cuellar, 1979).

The changes in plasma T levels during different phases of the male reproductive cycle in *C. versicolor* follow a pattern similar to that reported in other species of lizards with a prenuptial type of spermatogenesis (Arslan et al., 1978a; Courty and Dufaure, 1982; Bourne et al., 1986; Ando et al., 1990, 1992; Phillips and Millar, 1998; Tokarz et al., 1998). Though the average value of plasma T levels is highest during the breeding season, a variation in T levels is found among the individual lizards. Association of maximum T levels in individuals with stage IV testicular activity suggests that high levels of androgens may be needed for male–male combat, sexual display, and mating in *C. versicolor*.

Previous study on *C. versicolor* has shown a negative correlation between ovarian and fat body cycles. Also a lipolytic action of E2 on fat bodies has been reported (Shanbhag and Prasad, 1992). A negative correlation between plasma E2 and fat bodies observed in the present study supports previous findings in *C. versicolor* and in other species of lizards (Hahn, 1967; Greenberg and Gist, 1985; Guillette and Sullivan, 1985; Ramirez-Bautista et al., 2000). An inverse relationship between fat body mass and plasma T and testes mass observed in *C. versicolor* corroborates previous studies on other species of lizards (Guillette and Sullivan, 1985; McKinney and Marion, 1985; Diaz et al., 1994; Ramirez-Bautista et al., 2000). In *C. versicolor* the abdominal fat bodies do not have a role in spermatogenesis (Sharma and Shanbhag, 1992). Therefore, a reduction in fat body size (due to mobilization of lipids) and a rise in plasma T during the breeding season in *C. versicolor* seems to be associated with the events related to the behavioral repertoire related to reproduction.

In summary, in female *C. versicolor* with overlapping reproductive events such as vitellogenesis and gravidity, an inverse correlation is seen between plasma E2 and P. A rise in E2 levels is associated with the onset of recruitment of vitellogenic follicles. There is a good correlation among vitellogenic growth of follicles, oviduct mass, and plasma E2 levels. Following ovulation there is a fall in E2 and a rise in P levels. During eggshell formation P levels reach peak, but drop by mid-gestation and remain low until oviposition. In gravid females with vitellogenic follicles, E2 levels rise and P levels remain low. Therefore, it appears that high P levels are not needed for oviductal egg retention during late gestation. Plasma E2 and P levels are lowest during the postbreeding phase. In male *C. versicolor* changes in T levels are associated with high spermatogenetic activity.

**ACKNOWLEDGMENTS**

This work was supported by Grant No. SP/SO/C-16/96 from the Department of Science and Technology (DST), New Delhi awarded to B.A.S. and partially supported by UGC SAP-II, New Delhi. R.S.R. is thankful to DST for a Junior Research Fellowship.

**REFERENCES**


