
SHORT COMMUNICATIONS

Seasonal Changes of Steroid Levels in Blood Plasma of Three *Phodopus* Species (Mammalia, Cricetinae)

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Abstract—Seasonal change of the base plasma level of testosterone and cortisol in males and progesterone, estradiol, and cortisol levels in females were analyzed in three *Phodopus* species: *Ph. campbelli* (Campbell's hamster), *Ph. sungorus* (Dzungarian hamster), and *Ph. roborovskii* (Roborovskii dwarf hamster). Our results showed a significant difference in the seasonal plasma level of testosterone and cortisol in males and cortisol in females of all *Phodopus* species, though the rhythms of breeding activity were similar. The results are discussed in the context of adaptive differences between hormonal patterns of more evolutionarily modern species (Campbell's hamster and Dzungarian hamster) and the more evolutionarily old Roborovskii dwarf hamster, the phylogenetic position of which is currently under discussion.

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INTRODUCTION

Two *Phodopus* species, the Djungarian hamster (*Ph. sungorus*) and Campbell's hamster (*Ph. campbelli*), are popular research objects. The third species, Roborovskii dwarf hamster (*Ph. roborovskii*), recently became a research object (Feoktistova, 2008). The ecology of this species is poorly understood, so it is included in Kazakhstan's Government Regulation of Approval of Rare and Endangered Animal and Plant Species Index (2006) and in an annotated index of animal taxons and populations requiring monitoring of their environmental condition (The Red Book..., 2001).

The phylogenetic analysis of the Cricetinae subfamily revealed that Campbell's hamster and the Djungarian hamster are very close to each other (sister species). However, the difference between these two species and Roborovskii hamster has considerable mean values which indicate that this difference can be intergeneric (Neumann et al., 2006). Neumann and his colleagues (Neuman et al., 2006) suggest putting Roborovskii dwarf hamster in a separate genus, and associating Campbell's hamster and the Djungarian hamster in the genus *Cricetiscus*.

The study of seasonal changes in the hormone levels and breeding features can provide additional evidence to put the abovementioned species in two different genera.

Many physiological processes including reproduction are accompanied by hormone changes that are important for understanding many biological features of a particular species. However, the seasonal patterns of hormone changes in the abovementioned species have not yet been researched.

The purpose of our study is to analyze the characteristics of seasonal changes of testosterone, progesterone, and estradiol levels in males and females of three *Phodopus* species.

MATERIALS AND METHODS

The study was conducted from 2005 to 2009. Animals were kept in individual cages to study seasonal changes in hormone levels. All animals were housed on natural temperature and light cycles.

Dzungarian hamsters used for the experiments were bred from animals obtained in Khakasia in 1986–1989; Campbell's hamster were obtained by breeding animals obtained from the Ogii Nuur Lake area (Central Mongolia) in 2004; Roborovskii dwarf hamster were bred from animals obtained in Southern Tuva (Erzinsky district) in 1987–1989. Eight animals of each species and gender were used for the experiments, 48 animals in total. By the start of the experiments, the animals were 3–4 months old. All of them were eugamic and successfully bred before and after the experiments.

Animals were housed in standard plastic cages sized 22 × 9 × 15 cm and fed with a grain mixture (oats, millet, sunflower and wild plant seeds), bread, curds, and also vegetables (apples, carrots, beets, cabbage). Each animal received an abundant food supply.

Blood samples were obtained once a month at the same time of a day (from 9 to 10 a.m.) to exclude the influence of circadian hormone rhythms on the results. Blood samples (0.4–0.5 ml) were obtained from a tongue vein. This method (Graevskaya et. al., 1986) requires only 30 seconds for taking this amount

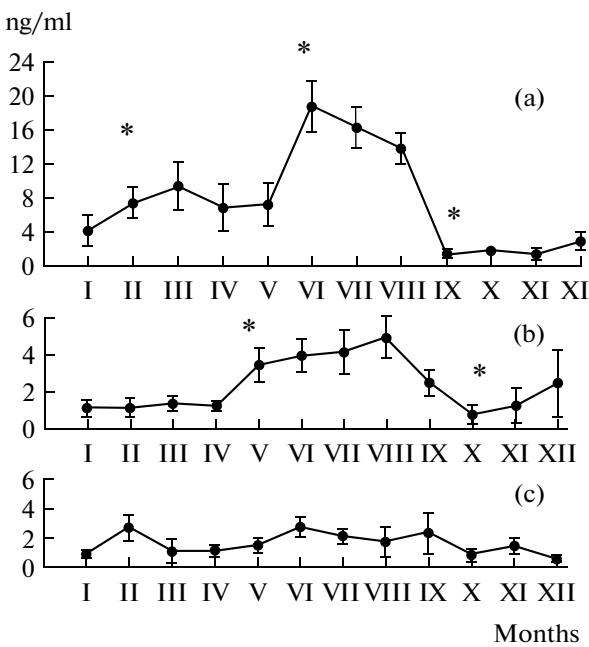


Fig. 1. Mean testosterone level ($M \pm SE$) in blood plasma (a) in male Roborovskii dwarf hamsters, (b) in male Campbell dwarf hamsters, and (c) in male Djungarian hamsters in different seasons of the year.

* indicates a significant difference between subsequent months ($p < 0.05$).

of blood, and allows estimating the base cortisol level. Blood samples were centrifuged at 6000 rpm to separate blood plasma. The obtained blood plasma was stored at -18°C before measurements. To estimate hormone concentrations heterogeneous immunoenzyme analysis was performed using a Multiscan EX microplate spectrophotometer (ThermoElectron Corporation, United States). Kits for evaluation of the testosterone, progesterone, and cortisol levels were purchased in Immunotek (Russia). Khema-Med (Russia) kits were used for evaluation of estradiol levels. The rate of testosterone antibody cross-reaction with other steroids was 9% for 5-dihydrotestosterone, 1% for 11-hydroxitestosterone, 1% for 5-androstene-3,17-diol, and less than 0.1% for the other tested steroids. The rate of cortisol antibody cross-reaction with other steroids was 6% for prednisolone, 0.9% for 11-desoxycortisol, 0.6% for corticosterone, and less than 0.1% for the rest of the tested steroids. Paired measurements were made to evaluate variation coefficient.

The phase of estrous cycle in females was determined after examination of vaginal smears. Only the data obtained from females in nonestrus were included in data analysis.

Statistical analysis was performed using Statistica 6.0 software. The Wilcoxon matched-pairs test was used to compare hormone levels during different sea-

sons of the year and in different species during a particular month.

RESULTS

The analysis of our data showed that male Roborovskii dwarf hamsters demonstrated a significantly increased level of testosterone in February, before the first peak of breeding (Fig. 1a), and then they had similar testosterone levels during the entire spring. The concentration of this hormone reached its maximum during the summer months, when the second peak of breeding activity occurred (Sokolov, Feoktistova, 1996; Meschersky, Feoktistova, 1999; Feoktistova, Meschersky, 1999, 2005; Feoktistova, 2008). After that, the base level of testosterone significantly decreased and remained low from September till January (Fig. 1a). However, there were males which had high levels of testosterone during the whole year. Their presence correlated with the appearance of litters in autumn and winter.

The average base level of testosterone in male Campbell and Djungarian hamsters was significantly lower ($p < 0.05$) than in Roborovskii dwarf hamsters during all seasons except autumn. In this period all three species demonstrated a similarly low level of testosterone.

The base plasma of testosterone in Campbell's and Russian hamsters increased neither before active spring breeding, which started in March, nor during spring breeding (Figs. 1b, 1c) (Sokolov, Feoktistova, 1998; Feoktistova, 2008). In summer, when breeding was the most active, the testosterone level in Campbell's hamster significantly exceeded its level in spring, but was lower than in Roborovskii dwarf hamsters (Fig. 1b). In Djungarian hamsters, there were no such observations. In this species, the testosterone level in males did not increase significantly, though it slightly exceeded its level in the autumn and winter periods (Fig. 1c).

In males of all three species, the base level of cortisol was relatively low, but there were differences in its seasonal dynamics.

The cortisol level in Roborovskii dwarf hamster was minimal in summer (during the maximal breeding rate period and testosterone peak), in October its level significantly increased and reached its peak in November (when the breeding rate decreased and testosterone level reached its minimal value), then the cortisol level was low from December to May (Fig. 2a). Campbell's hamsters demonstrated similar results (Fig. 2b). Low cortisol levels were found in the period of active breeding (from February to June). In August the cortisol level in males significantly increased, then its level remained high until December (when the breeding activity was decreased). In January the cortisol level decreased; this decrease became significant in February (Fig. 2b).

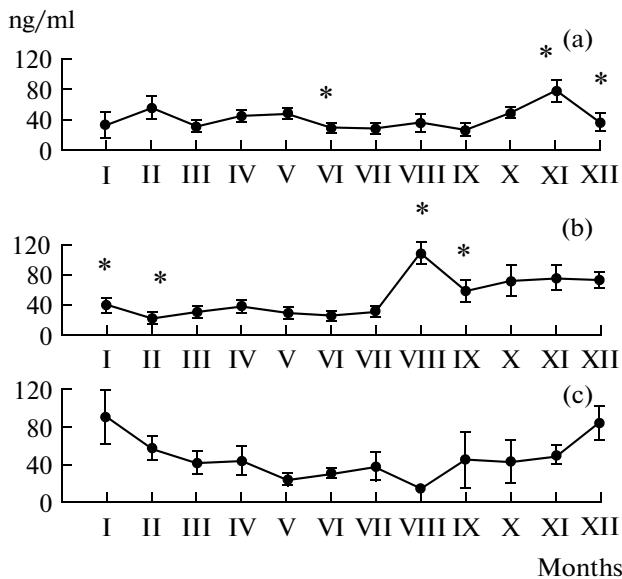


Fig. 2. Mean cortisol level ($M \pm SE$) in blood plasma (a) in male Roborovski dwarf hamsters, (b) in male Campbell dwarf hamsters, and (c) in male Djungarian hamsters in different seasons of the year.

* indicates a significant difference between subsequent months ($p < 0.05$).

Different results were obtained for the Djungarian hamster. There were no significant changes of cortisol level in males of this species during the year (Fig. 2c).

In females of all studied species, the testosterone level changed in a similar way. We observed two periods of increasing of the testosterone level during the year: before the start of the active breeding period and at its high point. However, each of the studied species demonstrated a special pattern of testosterone level changes. The female Roborovski dwarf hamster (Fig. 3a) had the maximal background level of testosterone in February; in March the concentration of this hormone significantly decreased. The second peak of testosterone level was observed from July to August, and then the testosterone level significantly decreased in September. In female Campbell dwarf hamsters (Fig. 3b), the first (insignificant) increase was observed in March, and the second, in June. In September the concentration of testosterone dramatically decreased. In female Djungarian hamsters (Fig. 3c), the testosterone level significantly increased in January and dramatically declined in April and May. The second peak of the testosterone level was observed in summer (June and July), then it significantly decreased.

Changes in the progesterone level also had similar patterns in all observed species. Its minimal values were discovered in autumn and winter (during the period of relatively inactive breeding). The progesterone level increase was observed in spring before the start of breeding; its concentrations remained relatively high until autumn. However, each studied spe-

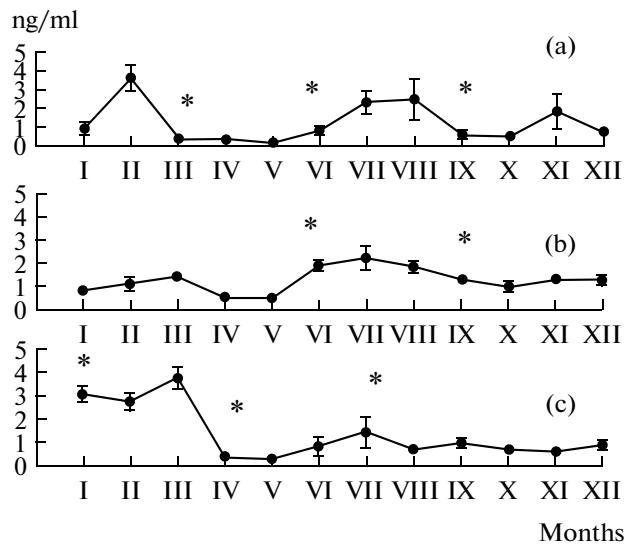


Fig. 3. Mean testosterone level ($M \pm SE$) in blood plasma (a) in female Roborovski dwarf hamsters, (b) in female Campbell dwarf hamsters, and (c) in female Djungarian hamsters during different seasons of the year.

* indicates a significant difference between subsequent months ($p < 0.05$).

cies demonstrated differences in the changes in the progesterone level. The minimal progesterone level in female Roborovskii dwarf hamsters (Fig. 4a) was observed from September till December, and the maximal level occurred from March until June. In female Campbell's hamsters (Fig. 4b), the base plasma level of progesterone started to rise in March, significantly increased in May, and remained high until August. The progesterone concentration declined in September. As in Roborovskii dwarf hamsters, a minimal level of this hormone was observed from September until December. In female Djungarian hamsters (Fig. 4c), the progesterone level significantly increased in February and remained high during spring and summer including July, decreased in August, and remained low until January.

The seasonal changes in the estradiol level were similar to progesterone, though some species had some differences. The observed concentrations of estradiol were often very low. In female Roborovski dwarf hamsters, the concentration of estradiol significantly increased in December, then slightly decreased in February and May, and in July it started to rise and reached its peak in July. In August the estradiol level declined and remained low during autumn (Fig. 5a). In female Campbell dwarf hamsters, the minimal estradiol level was observed in February and March, then it started to increase in April reaching its peak in May; the estradiol level remained the same until October and then decreased (Fig. 5b). In female Djungarian hamsters, the lowest level of estradiol was found in

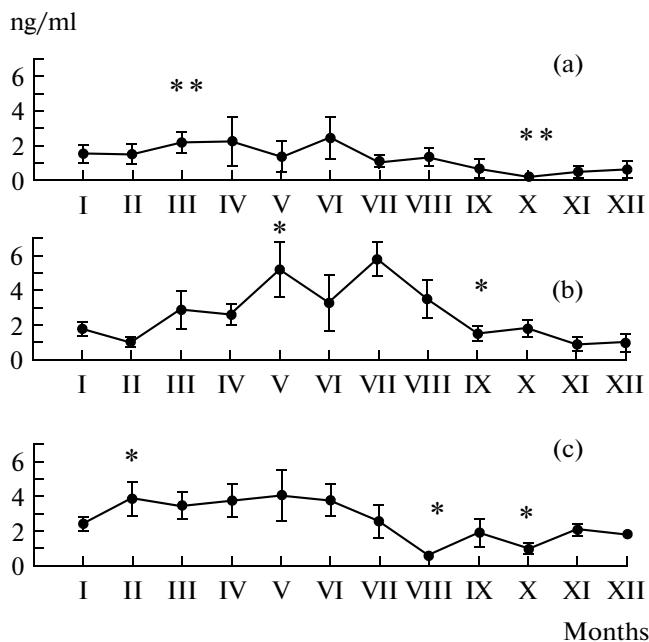


Fig. 4. Mean progesterone level ($M \pm SE$) in blood plasma (a) in female Roborovski dwarf hamsters, (b) in female Campbell dwarf hamsters, and (c) in female Djungarian hamsters in different seasons of the year.

* indicates a significant difference between subsequent months ($p < 0.05$).

autumn and the beginning of winter. It started to increase in January and reached its maximal values in March and July (with a decline in April). In August the estradiol level abruptly decreased (Fig. 5c).

Changes in the cortisol level were found to have the most significant interspecific difference.

In female Roborovski dwarf hamsters (Fig. 6a), the minimal cortisol level was found in April and May; then there was a significant increase in the cortisol concentration in July and a decrease in August. After that its level remained relatively stable until March. In female Campbell dwarf hamsters (Fig. 6b), the cortisol level did not change significantly during most of the year, although in November and December a significant and abrupt increase was observed. In female Djungarian hamsters (Fig. 6c), a decrease of cortisol level was observed in spring and summer (from May until August), and its minimal values were found in May and August. A significant increase was observed in winter (from December until January).

DISCUSSION

It is known that the secretion of sex steroid hormones is one of the key factors determining the success of reproduction and the structure of the energy budget of a specimen (Novikov, 2007, 2008) and immune response (Folstad, Karter, 1992; Mak et al., 2002).

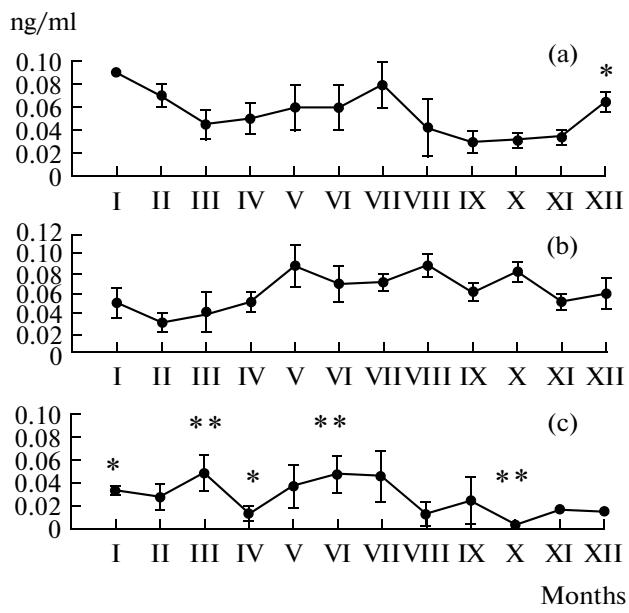


Fig. 5. Mean estradiol level ($M \pm SE$) in blood plasma (a) in female Roborovski dwarf hamsters, (b) in female Campbell dwarf hamsters, and (c) in female Djungarian hamsters in different seasons of the year.

* indicates a significant difference between subsequent months ($p < 0.05$).

To evaluate the mobilization ability of an organism, an adrenocortical response is usually used. The basal concentration of glucocorticoids reflects the total effect of previously received stress, and can be considered as an indicator of an animal's well-being (Novikov 2007, 2008).

Species with seasonal breeding, such as rodents, are known to have an increased level of testosterone during the period of breeding (short day length) (Zucker et al., 1980; Leonard, Ferkin, 1999).

We analyzed seasonal changes in the testosterone and cortisol basal plasma levels in males of three species and compared these changes with the breeding patterns of these species.

The low testosterone level in Campbell's and Djungarian hamsters during active breeding requires some explanation.

According to the hypothesis of immune handicap, androgens (particularly testosterone) on the one hand stimulate the development of secondary sexual characters and on the other hand suppress the mechanisms of humoral immunity (Folstad, Karter, 1992; Mak et al., 2002). Therefore, the high level of androgens in the blood plasma correlates with decreased resistance to diseases and parasitic infections.

The observed low base level of testosterone in the Campbell and Djungarian hamsters in the period of active breeding is evidence that they have adaptive mechanisms that allow successful breeding when the testosterone level is low, while preserving a high level of

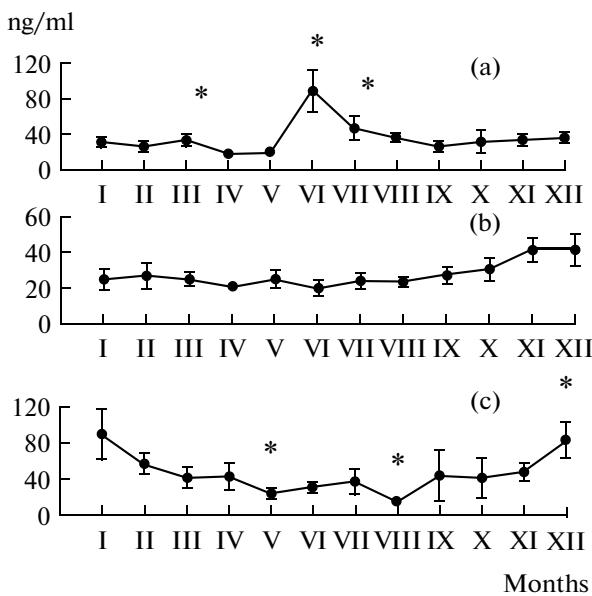


Fig. 6. Mean cortisol level ($M \pm SE$) in blood plasma (a) in female Roborovski dwarf hamsters, (b) in female Campbell dwarf hamsters, and (c) in female Djungarian hamsters in different seasons of the year.

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humoral immunity. However, the more evolutionary old Roborovski hamsters demonstrate a higher testosterone level during active breeding, which may indicate that their humoral immunity is less active.

Basically, the low base level of cortisol may indicate that the studied males are in good condition in different seasons. However, the significant decrease of this hormone level in the Djungarian and Campbell dwarf hamsters during the period of active breeding shows negative feedback of the adrenocortical and breeding activities, which can be adaptive because it increases the reproductive success and excludes the negative effect of glucocorticoids on reproductive function (Rogovin, Moshkin, 2007).

The lack of fundamental differences in steroid hormone levels and their seasonal changes in females of all studied species is due to features of reproductive system corresponding with the long period of breeding.

As in males, a low base level of cortisol in the studied females indicates their “well-being” during the year. A significant decrease in this hormone level in female Roborovski and Campbell dwarf hamsters during the period of active breeding indicates the negative feedback of adrenocortical activity and breeding, which enables an increase in reproductive success and excludes the negative effect of glucocorticoids on reproductive function (Rogovin, Moshkin, 2007). On the contrary, in female Roborovski dwarf hamsters the highest concentrations of cortisol are found during the period of active breeding, which indicates positive

feedback of adrenocortical activity and reproduction. This feature can be less adaptive and have a negative effect on success of reproduction.

Thus, our results demonstrate that in spite of similar dynamics of breeding, the three species studied have different seasonal features of hormone levels; the seasonal hormone response of both male (to a greater extent) and female Roborovski dwarf hamsters differs significantly from the other two species of the genus. Together with other factors (molecular genetic, morphological, karyological), this difference provides evidence that this species belongs to its own genus.

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