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Original article

Circannual changes in serum concentrations of thyroxine, calcitonin and parathormone in immature and mature red deer females (*Cervus elaphus*)

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Abstract

The aim of this study was to determine circannual changes in the serum concentrations of thyroxine, calcitonin and parathormone in mature and immature red deer females. Blood samples from 8 hinds were collected monthly for 26 months. Secretions of thyroxine and calcitonin showed circannual rhythms with significantly higher levels in the immature hinds compared to the mature animals ($p<0.05$). For thyroxine, the concentration was higher in the winter/spring period than in summer/autumn ($p<0.05$), while for calcitonin the concentration profile was the opposite ($p<0.05$). The concentration of parathormone was significantly higher in summer/autumn that in the other months of the experiment ($p<0.01$). These results may indicate that the hormones investigated may be involved in the regulation of seasonal reproductive activity and in processes contributing to entering puberty in red deer females.

Key words: thyroxine, calcitonin, parathormone, seasonality, puberty, red deer

Introduction

In recent years farm breeding of red deer (*Cervus elaphus*) has become more and more popular worldwide. Understanding the hormonal mechanisms that regulate reproduction rhythms in red deer in accordance to seasonality, which is strongly pronounced in a temperate climate zone, may contribute to improving breeding efficiency. Amongst various factors, change in the length of the day (photoperiod) is the most important source of information about the coming season, because, irrespective of changes in temperature or humidity, they occur continuously with the same regularity (Yasuo and Yoshimura 2009). Species which use the photoperiod to synchronize mating time with the season are divided into short- and long-day breeders. Red deer belong to the group of animals in which sexual activity begins in response to shortening day length (Anderson and Barrell 1998). In the temperate climate, the sexual activity in red deer females starts at the beginning of autumn (Scott et al. 2008). From the first ovulation, the ovaries remain active for about five to eight estrus cycles, each lasting about 18-21 days (McCorkell et al.

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2007). After that period, the seasonal *anestrus* begins (Asher 2011). Despite numerous studies in various animals on processes responsible for integrating signals indicating changing seasons with sexual activity, the precise mechanism is still unknown. Moreover, this kind of study is difficult due to the fact that species in human neighborhoods, as a consequence of domestication, lose their seasonal character of reproduction because their contact with natural environmental impulses is limited. The red deer may then be a reasonable animal model for this kind of research as it remains in contact with the natural environment and responds to all its impulses, in spite of being kept and bred in farms.

Various reports have implied the impact of hormones produced in the thyroid on seasonal regulation of sexual activity in red deer, but in this area, their role remains not fully explained (Nakao et al. 2008, Ashkar et al. 2010, Yoshimura 2010, Dittrich et al. 2011, Khan et al. 2011, Chu et al. 2012, Ikegami and Yoshimura 2012, Mutinati et al. 2013, Sechman 2013). Moreover, there are no reports indicating the circannual secretion profile of parathormone, produced in the parathyroid glands, in accordance to seasonality of breeding in red deer. In this study we aim to determine the circannual changes in the serum concentration of thyroxine, calcitonin and parathormone in mature and immature red deer females.

Material and Methods

The study was performed in 8 red deer females (*Cervus elaphus elaphus*) which at the beginning of the experiment were six months old. Blood samples (10 ml) were collected from the jugular vein, and because of the low harmfulness of this procedure, no analgesics were used. Blood serum samples obtained after centrifugation (10 min, 4000 rpm) were stored at -20°C until laboratory analysis. The samples were collected monthly over 26 months from November to December two years later.

The animals were kept on a farm located in Lubuskie voivodeship (Poland). The farm covers an area of 300 ha of lowlands near the Noteć river and Osiek lake. In the extensive breeding system about 1000 deer are farmed, grouped in 1-2 ha casements, with an access to fresh water. In summer the animals were fed a pasture diet and in winter supplementary feeding with silage, vegetables and cereals was applied. No mineral supplementation was used in the examined animals.

Based on the fact that in a temperate climate zone red deer reproductive activity begins in September/October (Scott et. al. 2008), the concentration of progesterone (P_4) was measured in all the samples taken in the first 15 months of the experiment to define the moment of entering puberty and to monitor the current status of the breeding season in females (Adam et al. 1985). Concentrations of total thyroxine (T4), calcitonin (CT) and parathormone (PTH) were evaluated in both years of the study to examine secretion intensity in reference to the sexual activity of the animals and seasonality. Concentrations of progesterone and thyroxine were evaluated by immunofluorescent method (TR-FIA) using Delfia Progesterone and Delfia T4 kits (Perkin Elmer, Turku, Finland). Concentrations of calcitonin and parathormone were evaluated by immunoenzimatic method (ELISA) using Bovine Calcitonin (CALCA/CALC) Elisa kit and Bovine Parathyroid Hormone (PTH) ELISA kit (Cusabio, Wuhan, China). All the analyses were conducted using a Wallac Victor 1420 unit (Wallac Oy; Turku, Finland).

Statistical analysis was performed with Statistica 10 software (Statsoft Polska, Kraków, Poland). The distribution of the results was evaluated with Shapiro-Wilk tests. The results were compared using repeated-measure analysis of variance, and the significance of differences between means was determined by Duncan's test. Analyses were conducted with significance levels of $p<0.05$ and $p<0.01$.

The experiment was approved by the local Ethics Committee in the Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin (agreement No. 29/2012).

Results

Progesterone

Changes in the serum concentration of progesterone in the red deer hinds in the first 15 months of the experiment are shown in Fig. 1. The mean concentration of P_4 in November and December (Nov. I and Dec. I) was 0.64±0.12 ng/ml and stayed at a similar level over winter and spring. In the summer period, a gradual increase in the concentration was observed, but the mean concentration did not differ significantly month by month. In contrast, from September to December (Sep. I to Dec. II) the concentration of P_4 remained at a mean level of 3.61±0.55 ng/ml and was significantly higher than in the previous months of the experiment ($p<0.01$). In January of the second year (Jan. II) the mean concentration of P_4 decreased to 0.94±0.34 ng/ml and was significantly lower than in autumn months ($p<0.01$).

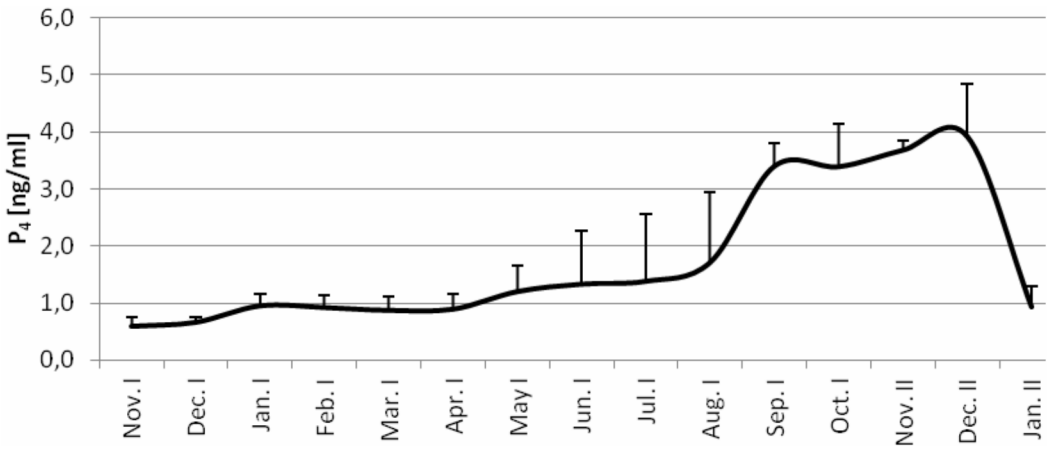

Fig. 1. Mean serum concentration of progesterone (P_4) in red deer hinds in the subsequent months of the experiment. Roman numerals after the month abbreviation refer to the year (Nov-Oct) of the experiment.

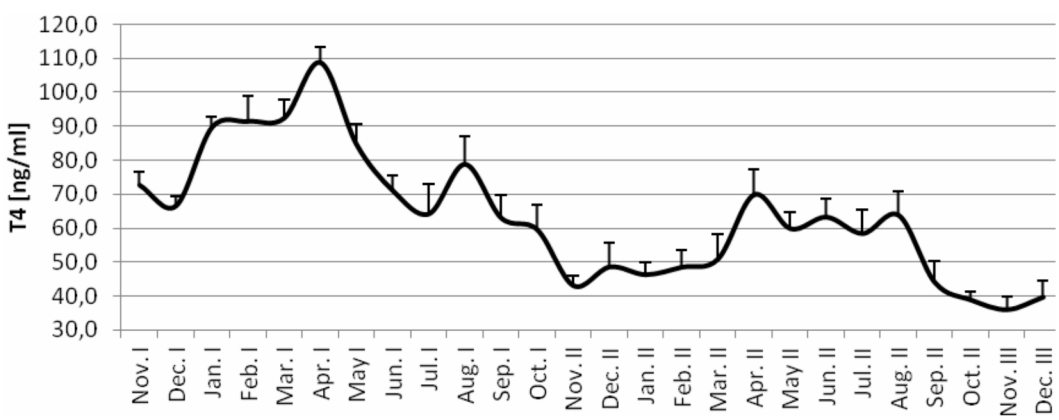

Fig. 2. Mean serum concentration of thyroxine (T4) in red deer hinds in the subsequent months of the experiment. Roman numerals after the month abbreviation refer to the year (Nov-Oct) of the experiment.

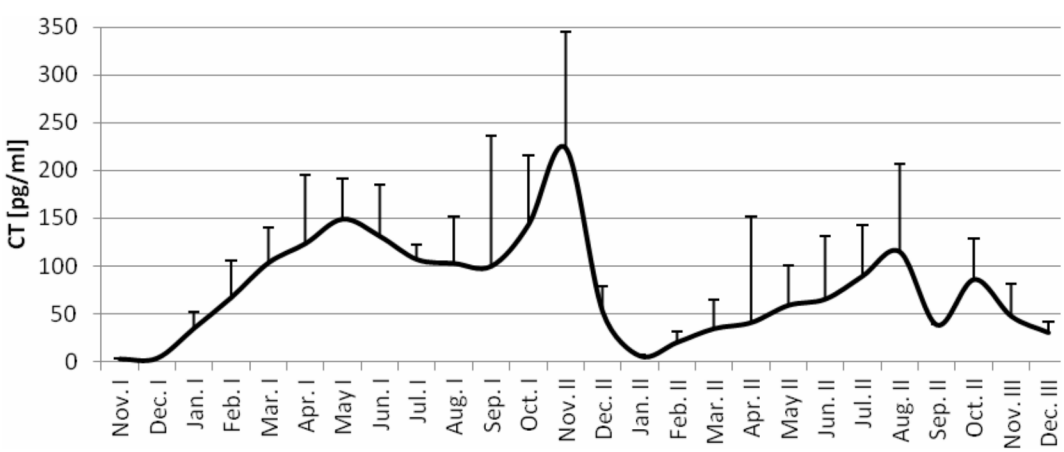

Fig. 3. Mean serum concentration of calcitonin (CT) in red deer hinds in the subsequent months of the experiment. Roman numerals after the month abbreviation refer to the year (Nov-Oct) of the experiment.

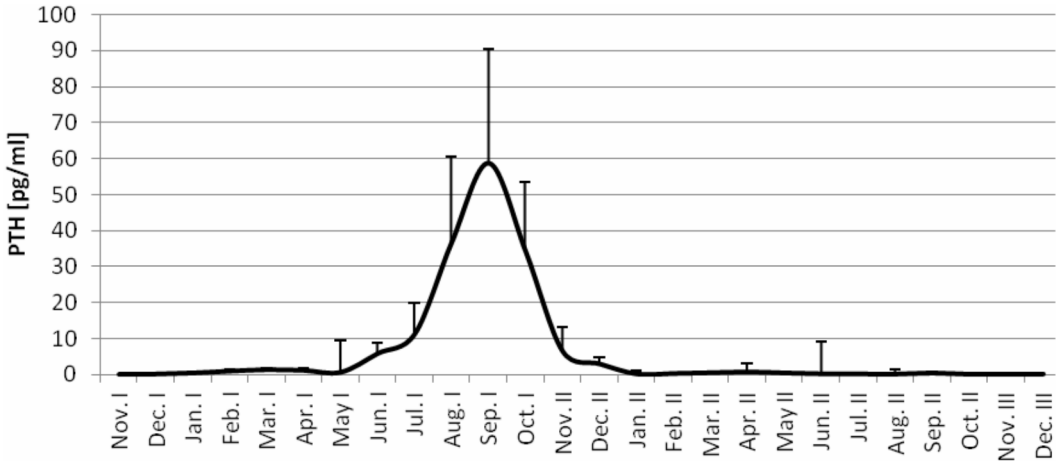

Fig. 4. Mean serum concentration of parathormone (PTH) in red deer hinds in the subsequent months of the experiment. Roman numerals after the month abbreviation refer to the year (Nov-Oct) of the experiment.

Thyroxine

Figure 2 shows changes in the serum concentration of total thyroxine in the red deer hinds during the 26 months of the experiment which began in November (Nov. I). Circannual dynamics of changes in the concentration of T4 in examined months was similar in both years of the study and was characterized by higher levels in the winter/spring period and lower levels during summer/autumn. The highest concentrations were observed in the first and second spring (Apr. I and Apr. II) at 108.78±4.52 ng/ml and 69.96±7.20 ng/ml, respectively. These concentrations were significantly higher than in the autumn months ($p<0.05$) during which the lowest level of T4 was found in the second and the third November (Nov. II and Nov. III).

The mean concentration of T4 differed significantly between the following years of the experiment ($p<0.05$). In the first year it reached 75.91±5.46 ng/ml and was significantly higher than in the second year (51.35±5.43 ng/ml).

Calcitonin

The dynamics of calcitonin concentration changes in the experiment was characterized by the lowest levels in winter and the highest in the summer/autumn period (Fig. 3). During the spring and summer months a gradual increase of calcitonin concentration was observed, with the highest value in November (Nov. II; 223.90±210.54 pg/ml). In the next year, the highest levels were noted in August (Aug. II; 114.83±91.45 pg/ml) and in October (Oct. II 85.81±42.39 pg/ml). The mean concentration observed during the winter months (42.01±21.18 pg/ml) differed significantly from those found in other months of the experiment ($p<0.05$).

Like thyroxine, the concentration of calcitonin was also significantly ($p<0.05$) higher in the first year (99.27±50.43 pg/ml) than in the second year of the study (52.53±40.41).

Parathormone

Changes in the concentration of parathormone over the period of the experiment are shown in Fig. 4. Analysis of concentration changes for the two years showed that a pronounced increase took place in the period from June (Jun. I) to November (Nov. II) in the animals second year of life. The mean concentration in this period was 25.65±21.23 pg/ml, with the maximum in September (Sep. I; 58.81±31.63 pg/ml) and was significantly higher than the concentrations observed in the other months of the study ($p<0.01$). Since December in the second year of experiment until the end of the study, the concentration of parathormone remained at a low and constant level (0.61±0.92 pg/ml). Significant differences were shown between mean concentration of parathormone in the first (12.27±7.93 pg/ml) and the second (0.61±0.92 pg/ml) year of the experiment ($p<0.01$).

Discussion

The results obtained in this study suggest that the examined hinds entered puberty during the second autumn after birth. This is evidenced by the increased concentration of progesterone, which indicates the secretory activity of the corpora lutea in the ovaries. In red deer hinds, as in other species females, the rise in

progesterone concentration demonstrates the luteal activity (Asher et al. 2011). According to Asher and Cox (2013), hinds usually enter puberty at the age of 18 months, and in favorable conditions, up to two months earlier. Our results confirm that red deer females under farm maintenance may enter puberty at the age of 16 months. In general in this species, puberty occurs during the second autumn after birth, thus a female can participate in the rut in the same year (Asher et al. 2011). The changes in progesterone concentration may indicate that the rutting season in the examined hinds lasted from September to December. Similar results about the beginning of the rutting season in farmed hinds were obtained by Asher et al. (2011), while McCorkell et al. (2007) described the rutting season as lasting about 140 days.

The opening and closing of the rutting season in short-day breeders is controlled by many endo- and exogenous factors. The main hormone involved in the regulation of the beginning of the rutting season is melatonin, while iodothyronines like thyroxine are considered in the mechanisms controlling entrance into the seasonal *anestrus* (Nakao et al. 2008, Yoshimura 2010). The results obtained in this work confirm that thyroxine secretion shows circannual rhythms, characterized by higher concentrations in winter/spring than in the summer/autumn period (Webster et al. 1991, Abecia et al. 2005). According to some authors, closing of the rutting season is a consequence of an increase in thyroxine concentration observed at this time. This theory is supported by numerous studies in ewes and red deer, in which a thyroidectomy elongated the rutting season (Webster et al. 1991, Shi and Barrell 1992, 1994, Thrun et al. 1997, Anderson and Barrell 1998, Anderson et al. 2003).

However, the results relating to the entrance to *anestrus* obtained with the use of pharmacologically induced hypothyreosis are not that unambiguous (Hernandez et al. 2003, Błaszczyk 2011). Moreover, it has to be considered that an increased concentration of thyroxine during the winter period may in part be caused by the need to adjust metabolic processes, like mobilization of energy reserves and intensification of thermoregulation for winter (Todini et al. 2007, Paul et al. 2008, Novoselec et al. 2009). In the first year of the hinds life, the dynamics of thyroxine concentration changes was similar to the annual rhythm observed in older animals, yet the concentrations found in the younger animals were much higher. These results correspond with data in other species, and show that the concentration of thyroxine in blood serum depends, among others, on age (Lucaroni and Todini 1989, Hamr and Bubenik 1990, Dawson et al. 1996). It also cannot be excluded that the differences are caused by the puberty entrance in the examined females.

Assuming that thyroidectomy blocks the entrance to the seasonal *anestrus*, and pharmacologically induced hypothyreosis cannot prevent the beginning of this period, we cannot exclude that the control of breeding seasonality is held by other hormones synthesized right in the thyroid (calcitonin) or in its close proximity, in the parathyroid glands (parathormone). A seen in this work, changes in the concentration of calcitonin undergo seasonal rhythms, which corresponds to the results obtained by other authors (Chao et al. 1984). Like in thyroxine, the values observed were higher in the younger females, therefore we may suppose that the age of the animal affects calcitonin secretion. The role of calcitonin in regulating breeding seasonality cannot be excluded, as supported by the fact that in both the first and second year of the study, a rapid increase in the concentration of this hormone was found in the early period of sexual activity. In turn, in the period preceding the entrance to *anestrus* in both years of the experiment, an evident decrease in calcitonin concentration was observed. Therefore we cannot exclude that the results obtained using a thyroidectomy are the result of depriving the organism of the physiological effects of not only iodothyronines but also calcitonin.

In case of parathormone, no circannual rhythms in the secretion were observed. Nevertheless, the differences in the concentration found between immature and mature hinds are an argument to indicate the role of this hormone in the regulation of reproductive processes. It is interesting that the highest concentration of parathormone was observed in September in the second year of life, which is at the time that the females entered puberty. In turn, during the following year of life no increased concentration of parathormone was observed. This suggests that this hormone may take part in the processes contributing to entering puberty.

Previous knowledge about the role of parathormone refers to mineral management in the body (Nussey and Whitehead 2001, Trelińska and Bodalski 2007). For this reason, studies on this hormone in red deer were conducted in the context of the seasonal growth of antler in males (Faucheux et al. 2002, Price and Allen 2004), in which elements like calcium, magnesium and phosphorus play the crucial role (Landete-Castollejos et al. 2012). Regarding our surprising results, further studies on the potential role of parathormone in the regulation of reproduction processes in this species are needed.

In conclusion, considering the results obtained, we may suppose that in red deer hinds in farm conditions the breeding season lasts from September to December. The profile of changes in serum concentrations of thyroxine and calcitonin confirms circannual rhythms

of thyroid hormone secretions, which may imply the role of not only thyroxine but also calcitonin in the regulation of breeding seasonality. Moreover, differences in the concentrations of thyroxine, calcitonin and parathormone revealed between immature and mature hinds suggest the influence of these hormones on processes leading to puberty in red deer females.

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