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Steroid hormones in peripheral blood plasma and androgen receptors in testis and epididymis of roe deer male (*Capreolus capreolus*) during the reproduction season

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ABSTRACT

The roe deer (*Capreolus capreolus*) is a seasonally breeding species. The regression of the gonads and the inhibition of the process of spermatogenesis during the winter, the regeneration of the gonads during the spring and the occurrence of the highest level of spermatogenesis during the summer are observed in mature males. A radioimmunological analysis of the steroid hormone levels in the peripheral blood of male roe deer, measured at the beginning (May), at the peak (July/August) and at the end (September) of the reproductive season, demonstrated that the highest levels of progesterone, androstenedione, testosterone, and oestradiol coincided with the peak of reproductive activity. In addition, an immunohistochemical localisation of androgen receptors (AR) in the testis and epididymis during the same study periods showed that the highest immunoreactivity of the target cells occurred at the peak of the reproductive season (July/August). The results of the study indicate that all of the sex hormones (not only testosterone) may play a significant role in the regulation of reproductive processes. Increasing levels of steroid hormones (especially the androgens) induce the expression of androgen receptors in the target cells of the testis and epididymis, ensuring their normal function and maintenance.

1. Introduction

Numerous mammal species living in temperate climates are characterised by seasonal breeding, an adaptation to annual changes in the habitat. Mature males demonstrate synchronous cycles of growth in the gonads and epididymides during the reproductive season and the subsequent regression of these organs (Bronson and Heidemann, 1994). The complete inhibition of the process of spermatogenesis during the winter and the resumption of this process in May (middle spring) is observed in male roe deer, whereas the highest level of spermatogenesis coincides with the period of rut in females (middle summer). This period normally begins in mid-July and ends in mid-August (Semperé et al., 1998; Göritz et al., 2003). These changes are accompanied by morphometric, structural and functional changes in the testes (Schön et al., 2004) and epididymides (Schön and Blottner, 2009). Cyclic transformations are primarily controlled by the hypothalamic–pituitary–gonadal axis endocrine and influenced by endogenous rhythms and by photoperiod (Asher et al., 1994). Indicate that the amounts of LH and FSH secreted by the pituitary gland began to increase in January and to reach their maximum values in March. They then decrease until April and finally maintain a constant level until December (Roelants et al., 2002). Increases in blood plasma levels of gonadotropins in March induce the activation of the differentiation of Leydig cells. This event initiates the synthesis of testosterone and of growth factors. In mature male roe deer, the expression of INSL3 protein, considered a marker of mature Leydig cells, is highest one month after the peak in the level of gonadotropins

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(Hombach-Klonisch et al., 2004). Measurable amounts of 17α-hydroxylase, i.e. the initial steroidogenic enzyme in the <DELTA>5 pathway where progesterone, androgen and oestrogen are formed (Conley and Bird, 1997). The level of plasma testosterone increases beginning in April, then decreases slightly in June, and finally shows a rapid increase. Its highest level occurs simultaneously with the highest level of activity of the process of spermatogenesis and the performance of typical male mating behaviour. In males, the peak in plasma testosterone coincides with the short rut period (lasting from mid-July to mid-August) (Blottner et al., 1996). The concentration of testosterone then decreases rapidly. Beginning in October, it remains at a low level. The fluctuations in the level of testosterone in the testis (the testosterone concentration per unit testis weight) throughout the year are very similar to the variations in the concentration of that hormone in the peripheral blood (Roelants et al., 2002). It was also shown that oestrogens are involved in the seasonal regulation of the process of spermatogenesis and the maturation of spermatozoa in the male roe deer. This finding is confirmed by the expression of cell-specific (Sertoli cells, spermatogonia, round spermatids, epithelium of the ductuli efferentes, epithelial cells of the epididymis caput) oestrogen receptors (ERα and ERβ) and also by the presence of aromatase in the testis and epididymis during and after the reproductive season (Schön and Blottner, 2008). So far, no data concerning the location of androgen receptors in the reproductive organs of male roe deer and no information on changes level of steroid hormones (with the exception of testosterone) during the reproductive season. Therefore, the aim of this study was to analyse whether the progesterone, androstenedione and oestradiol concentrations from May through September show fluctuations similar to those shown by testosterone. Furthermore, the study aimed to determine whether the sensitivity of the tissues of the testis and epididymis to androgens (analysis immunohistochemical detection of the androgen receptor) is subject to changes during that period.

2. Materials and methods

The material for the study was collected from 36 mature male roe deer (bucks) aged from 4 to 8 years (defined based on dentition) during hunts occurring from 2005 through 2009 in the Podkarpacie region (50°1′N; 22°0′E) of Poland in central Europe during the following three periods:

(1) pre-rut period – from 10 to 31 May,
(2) rut period – from 15 July to 15 August (oestrus period in females according to Semperé et al., 1998),
(3) post-rut period – from 10 to 31 September (when females were no longer in oestrus).

The number of animals for each experimental period was 12. All animals lived in the wild.

3. Determination of the concentration of steroid hormones

Blood from left ventricle of the heart was collected in heparinised test tubes, transported to the laboratory (in a vacuum bottle at 4 °C) within 2 h after hunting and centrifuged for 15 min at 1500 × *g*; the serum obtained was frozen at −20 °C until the concentration of steroid hormones was determined.

The concentrations of total progesterone, androstenedione, testosterone and oestradiol were measured with the radioimmunoassay method (RIA) using commercial kits (BioSource Europe S.A, Belgium). For total progesterone: the sensitivity of the method was 0.05 ng/ml, the intra- and interassay CVs were 4.1% and 8.6% respectively, the assay range from 0.1 to 36 ng/ml. The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively: 17-α-hydroksyprogesteron 1.5%, 20-β-dihydroprogesteron 3.27%, 5-α-pregnan-3,20 dion 3.51%, 20-α-dihydroprogesteron 0.03%, pregnenolon 0.38%, testosteron 0.03%, androstenedione 0.12%, oestradiol < 0.0012%.

For total androstendione: the sensitivity of the method was 0.03 ng/ml, the intra- and interassay CVs were 4.5% and 9.0% respectively, the assay range from 0.1 to 11 ng/ml. The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively: 17-α-hydroksyprogesteron 0.08%, kortyzol 0.1%, testosteron 0.24%, dehydroepandrostendion 0.01%.

For total testosterone: the sensitivity of the method was 0.05 ng/ml, the intra- and interassay CVs were 4.6% and 6.2% respectively, the assay range from 0.1 to 17 ng/ml. The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively: dihydrotestosterone 0.31%, androstenedione 0.28%, 17-β-estradiol 0.004%, progesterone 0.01%.

For total oestradiol: the sensitivity of the method was 2 pg/ml, the intra- and interassay CVs were 5.9% and 10.1% respectively, the assay range from 10 to 3551 pg/ml. The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively: estrone 1.8%, estriol 1.2%, testosterone 0.0012%, progesterone 0.002%, androstenedione 0.0011%, 17α estradiol 0.5%.

4. Histological specimens

The testes and epididymides were collected immediately after hunting, placed in PBS (phosphate-buffered saline pH 7.4) and transported to the laboratory at 4 °C within 2 h after removal from the animals. Small pieces of the testis and epididymis (from caput and cauda) were cut and fixed in 4% paraformaldehyde for 24 h, dehydrated and embedded in paraffin. The tissues were cut into 5 μm thick sections.

5. Immunohistochemical reaction

Paraffin sections of the testes and epididymides (from caput and cauda) were deparaffinised and rehydrated. For detection, an antigen retrieval was performed by boiling the slides in 10-mM citrate buffer (pH 6.0) for 2 min. To block endogenous peroxidase activity, the slides were incubated in TBS (0.05 M Tris–HCl plus 0.15 M NaCl, pH 7.6) with 0.3% H_2O_2 addition for 20 min. To block non-specific binding, the sections were incubated for 30 min with 5% normal goat serum (NGS). In addition,