Annual profiles of reproductive hormones in peripheral plasma of the male roe deer (*Capreolus capreolus*)

D. Schams and D. Barth*

*Lehrstuhl für Physiologie der Fortpflanzung und Laktation, Technische Universität München,
D-8050 Freising-Weihenstephan, and MSD Research Laboratories, Sharp & Dohme,
D-8201 Lauterbach, West Germany*

Summary. Blood samples were collected from 13 roebucks 1 to 3 times per month during several years to study the relationship between season and concentrations of LH, FSH, testosterone and prolactin. Plasma values of all of these hormones began to increase during the second half of February. LH, FSH and testosterone levels reached a plateau during April. Highest average levels of LH and testosterone were observed before the start of the rutting season (mid-July–mid-August) and steadily declined thereafter. In contrast to LH and testosterone, FSH concentrations decreased 6 weeks earlier, during June, indicating a modulated secretion pattern. Prolactin steadily increased until the beginning of July and then decreased.

Introduction

The roe deer, *Capreolus capreolus*, is a seasonal breeder with a rutting season from the middle of July to the middle of August. This is in contrast to other cervids, such as the red deer (*Cervus elaphus*), caribou (*Rangifer arcticus*), white-tailed deer (*Odocoileus virginianus*) and black-tailed deer (*Odocoileus hemionus*), in which the rutting season takes place during the fall. In the roe deer parturition occurs in May or June after a gestation length of 300 days (Stieve, 1950; Short & Hay, 1966; Wandeler, 1975). The roe deer is the only ungulate to exhibit delayed implantation, as first described by Ziegler (1843) and Bischoff (1854). The roe deer embryo remains in diapause for 5 months (Stieve, 1950; Short & Hay, 1966; Aitken, 1981). The roe deer appears to be monoestrous. If the corpus luteum is formed, even in the non-pregnant animal, it remains active during the greater portion of the reproductive period (Hoffmann, Barth & Karg, 1978; Schams, Barth & Karg, 1980). Marked changes in testicular size, hormone secretion, behaviour and antler cycle during the year are reported (Stieve, 1950; Short & Mann, 1966; Barth, Giménez, Hoffmann & Karg, 1976; Sempéré, 1978; Sempéré & Boissin, 1981). After mid-February the size of the testis and testosterone concentrations increase until the beginning of the rut and decrease afterwards. Spermatogenesis is completed by May. The onset of the rut about 2 months later is presumably due to the occurrence of oestrus in the doe. These endocrine changes accord with the seasonal changes in antler growth. Antlers begin to develop in January. Shedding of the velvet occurs soon after the testicular testosterone concentrations have risen in the spring, and casting of the antlers in late fall coincides with very low testosterone levels. No reports are available for changes in prolactin and FSH of roebucks. The purpose of the present study was to determine the relationship between season and plasma concentrations of LH, FSH, testosterone and prolactin. Part of the testosterone data has been published by Barth *et al.* (1976).

Materials and Methods

Experimental animals and blood sampling

The study was made on 13 bucks from 1973 until 1977. Nine bucks were born in the wild in 1972 and trapped at 1–20 days of age. After a period of bottle feeding and following the acceptance of roughage, they were housed in enclosures within their natural environment. The other 4 animals were born in captivity in 1975 (3) and 1976 (1). The housing, nutrition and management of the animals have been described previously (Barth *et al.*, 1976). Blood was collected from adult bucks (2–5 years old) 1–3 times monthly for the whole year (9 animals, 15 cycles) or only during certain periods (January–June; 7 animals, 9 cycles; or April and May–July; 7 animals, 7 cycles). Throughout the experiments, blood was withdrawn from the jugular vein between 09:00 and 10:00 h by venepuncture. Blood plasma was obtained within 1 h after bleeding and stored frozen until assayed.

Hormone concentrations in samples obtained during half-month periods were grouped together (mean $\pm$ s.d.) and the Wilcoxon range test was used to compare groups.

Hormone determinations

The concentrations of LH, FSH, prolactin and testosterone were evaluated radioimmunologically as described by Schams & Karg (1969a), Schams & Schallenberger (1976), Schams & Karg (1969b) and Karg *et al.* (1976) respectively.

LH. The assay for LH was an homologous system for bovine LH. The antiserum, raised in a rabbit, showed no cross-reaction with other pituitary hormones. For radioiodination of pure bovine LH the chloramine-T method was used. Possible unspecific, interfering influences of roe deer plasma were eliminated by adding a constant amount of roe deer plasma (0.1 ml, 1:9 v/v diluted with buffer) with a low content of LH to each tube. Total incubation volume of the assay was 0.5 ml when using 0.2 ml of unknown sample. Sensitivity of the assay was 0.05 ng/tube. The intra-assay coefficient of variation (CV) calculated from a mean value of 20 parallel tubes for 2 different plasma samples (0.15 ng and 0.4 ng/0.2 ml), was an average of 5.8%. The inter-assay variation, expressed as the mean of the variation coefficients of 4 different roe deer samples, was 11.5%.

FSH. A heterologous system with pure ovine FSH for labelling (lactoperoxidase method) and a guinea-pig antiserum against ovine FSH was used. The slight cross-reaction with bovine LH and TSH was eliminated by adding a constant amount of LH and TSH to each tube within the assay. Furthermore, to avoid unspecific interfering influences, a constant amount of roe deer plasma (0.1 ml, diluted 1:9 v/v with buffer) was added to each tube. Sensitivity of the assay was 2.5–5 ng/tube. The intra-assay CV, calculated from a mean value of 15 parallel tubes of 2 different plasma samples (25 and 50 ng/tube), was an average of 6.5%. The interassay variation, expressed as the mean of 3 different roe deer samples run in the assay, was 13.6%.

Prolactin. The assay for prolactin was an homologous system for bovine prolactin. The antiserum, raised in a rabbit, showed no cross-reaction with other pituitary hormones. From radioiodination of pure bovine prolactin the lactoperoxidase method was used. Sensitivity of the assay was 0.25 ng/ml. The intra-assay CV calculated from control samples run at the beginning, in the middle and at the end of the assay was 8.4%. The interassay CV was 16.3%.

Cross-reaction and parallelism with the bovine standards were checked by inhibition curves of a pituitary extract from roe deer pituitaries and plasma samples of roe deer containing higher concentrations of endogenous hormone; these were parallel, as shown, for example, for LH and FSH in pituitary extracts by Schams *et al.* (1980) and for prolactin in Text-fig. 1. In all assays (LH, FSH and prolactin) after incubation for 24 h at 4°C, labelled hormone (about 10 000 c.p.m.) was added to each tube. Separation of bound and free hormone was performed by means of the double-antibody methods. Bovine pituitary preparations were used as the reference standards for LH-DSA (prepared in our laboratory, biological activity of $1.0 \times \text{NIH-LH-S}_1$), for FSH (NIH-FSH-B₁, biological activity $0.49 \times \text{NIH-FSH-S}_1$) and for prolactin (NIH-P-B₃, biological activity 24.1 i.u./mg).

Text-fig. 1. Inhibition curves of bovine prolactin (●) compared to inhibition curves of a pituitary extract obtained from roe deer pituitaries (○).

Testosterone. As within the method originally described, specificity was improved by using a highly specific antiserum raised against testosterone-11-hemisuccinate-BSA. There was a negligible cross-reaction with androstanolone and androstendione. Sensitivity of the assay was about 50 pg/ml. The inter-assay CV, expressed as the mean of 3 different samples containing different amounts of testosterone, was in the order of 7.5–12%.

Results

The hormone values obtained from the 13 bucks are summarized in Text-fig. 2.

LH

Concentrations began to increase after the first half of February (statistical significance was obtained from the first half of February to the second half of March, $P < 0.05$) and reached a plateau during April. The highest average value was measured before the rutting season started with a steady decrease ($P < 0.05$ at the end of August compared with the highest value during July) afterwards. Basal levels were measured from September onwards.

Testosterone

Concentrations were very low at the beginning of February and then increased ($P < 0.05$) during the second half of February, reaching a plateau during April. A further increase was observed during July, at the beginning of the rut. During August, testosterone levels decreased ($P < 0.05$ at the second half of August compared to July) with a further decline thereafter until December.

FSH

Like those of LH and testosterone, FSH values were elevated during the second half of February. Following this increase a slight decrease occurred and then a further continuous increase until the second half of April (which was significant compared to February) when a plateau was

Text-fig. 2. Concentrations of LH, testosterone, FSH and prolactin in peripheral plasma of 13 roebucks during the annual cycle. Values are mean $\pm$ s.d. for samples throughout half month intervals grouped together. The numbers indicate the number of samples during that period. The solid bar denotes the rutting season.

reached. After a maximum average value in the second half of May the FSH levels in June decreased steadily to the lower basal concentrations observed at the beginning of the rut and remained low during the rest of the year.

Prolactin

Like the other hormones, there was an elevation during the second half of February with a continuous increase ($P < 0.05$ compared with the first half of February) until June. The highest average value was observed at the beginning of July. Prolactin levels decreased continuously thereafter.

Discussion

The high standard deviation of the mean values for all the hormones measured can be partly explained by the infrequent samplings. If we assume that the hormones are secreted in a pulsatile pattern as demonstrated for LH in rhesus monkeys by Knobil (1974) and in wild animals like the red deer by Lincoln & Kay (1979), we can expect to have a large amount of variation among samples taken infrequently. This is especially true for prolactin since handling of the animals for blood sampling may have a stress effect too.

A seasonal rhythm was observed for all the hormones measured. The profile for testosterone agrees with reports in the literature (Short & Mann, 1966; Barth *et al.*, 1976; Sempéré, 1978; Sempéré & Boissin, 1981) and corresponds to those for other male cervids in which the rutting season occurs later, e.g. red deer (Lincoln, Youngson & Short, 1970), white-tailed deer (McMillen, Seal, Keenlyne, Erickson & Jones, 1974) and Columbian black-tailed deer (West & Nordan, 1976).

The increase in testosterone concentrations during February/March and the remaining high values are closely related to the events of spermatogenesis (Short & Mann, 1966). While spermatogenesis in the roebuck is more or less completed by May, the testosterone rise just before and at the beginning of the rutting season may be correlated to sexual behaviour. The LH pattern associated with the pattern of testosterone secretion supports the assumption that LH stimulates the Leydig cells to produce testosterone. The LH decrease at the beginning of the rutting season precedes the drop in testosterone values. This may be explained by the negative feedback action of testosterone on LH synthesis and release. A decrease of LH content in pituitaries of roebucks during the rut was demonstrated by Brüggemann, Adam & Karg (1965).

The increase in FSH secretion followed, in general, the increase in LH and testosterone secretion, suggesting that FSH and LH secretion are closely related in stimulating spermatogenesis and androgenesis. However, FSH values decreased about 6 weeks earlier than those of testosterone and LH and coincided with the completion of spermatogenesis (Short & Mann, 1966). This indicates modulation of FSH secretion by other factors, such as inhibin, as suggested for the rat (Steinberger & Steinberger, 1976). Lincoln & Short (1980) have stressed the natural discrimination of LH and FSH regulation in seasonally breeding animals by changes in pulse frequency of LH.

The role of prolactin in roebucks is unknown. A role in testicular function as demonstrated for rats and mice (Bartke & Dalterio, 1976; Bartke, Goldman, Bex & Dalterio, 1977) needs further elucidation. A marked seasonal variation in prolactin concentrations was observed, as in other ruminants such as cattle (Karg & Schams, 1970; Schams & Reinhardt, 1974), rams (Ravault, 1976) and white-tailed deer (Mirarchi, Howland, Scanlon, Kirkpatrick & Sanford, 1978). The prolactin levels increased with increasing daylength and declined with decreasing daylength. The parallel elevation of all the hormones measured during the end of February is presumably related to the initiation or resumption of sexual activity in response to increasing daylength. This is in contrast to other cervids in which sexual activity is stimulated by a decrease in daylength.

The endocrine changes observed in this study are in accord with the seasonal changes in antler growth, which are known to be under endocrine control. Testosterone especially is known to play an important part in controlling the antler growth cycle (Wislocki, Aub & Waldo, 1947). Shedding of the velvet occurs soon after the rise of testosterone in March (Short & Mann, 1966; Barth *et al.*, 1976; Sempéré & Boissin, 1981) and casting of the antlers occurs in November or December, when very low testosterone levels are measured. In white-tailed deer, treatment with an antiandrogenic compound completely blocked the mineralization process of the bone matrix and the antlers persisted in growing throughout the whole year (Bubenik, Bubenik, Brown & Wilson, 1975).

We are most grateful to the National Institutes of Health, Bethesda, USA for generous gifts of bovine pituitary standard preparations (NIH-P-B₃ and NIH-FSH-B₁). We thank Mrs Kürzinger for expert assistance.

References

- Aitken, R.J. (1981) Aspects of delayed implantation in the roe deer (*Capreolus capreolus*). *J. Reprod. Fert., Suppl.* **29**, 83–95.
- Barth, D., Giménez, T., Hoffmann, B. & Karg, H. (1976) Testosterone-konzentrationen im peripheren Blut beim Rehbock (*Capreolus capreolus*). Saisonale und rhythmische Veränderungen sowie Beziehungen zu einigen Verhaltensweisen und anderen saisonabhängigen Parameter n. Z. *Jagdwiss.* **22**, 134–148.
- Bartke, A. & Dalterio, S. (1976) Effects of prolactin on the sensitivity of the testis to LH. *Biol. Reprod.* **15**, 90–93.
- Bartke, A., Goldman, B.D., Bex, F. & Dalterio, S. (1977) Effects of prolactin (PRL) on pituitary and testicular function in mice with hereditary PRL deficiency. *Endocrinology* **101**, 1760–1766.
- Bischoff, Th. L.W. (1854) *Entwicklungsgeschichte des Rehes*. J. Rickers Buchhandlung, Giessen.
- Brüggemann, J., Adam, A. & Karg, H. (1965) ICSH-Bestimmungen in Hypophysen von Rehböcken (*Capreolus capreolus*) und Hirschen (*Cervus elaphus*) unter Berücksichtigung des Saisoneinflusses. *Acta endocr., Copenh.* **48**, 569–580.
- Bubenik, G.A., Bubenik, A.B., Brown, G.M. & Wilson, D.A. (1975) The role of sex hormones in the growth of antler bone tissue. *J. Exp. Zool.* **194**, 349–358.
- Hoffmann, B., Barth, D. & Karg, H. (1978) Progesterone and estrogen levels in peripheral plasma of the pregnant and non-pregnant roe deer (*Capreolus capreolus*). *Biol. Reprod.* **19**, 931–935.
- Karg, H. & Schams, D. (1970) Prolactin levels in bovine blood under different physiological conditions. In *Lactation*, pp. 141–143. Ed. I. R. Falconer. Butterworth, London.
- Karg, H., Giménez, T., Hartl, M., Hoffmann, B., Schallenberger, E. & Schams, D. (1976) Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral plasma of bulls: Levels from birth through puberty and short term variations. *Zentbl. VetMed. A* **23**, 793–803.
- Knobil, E. (1974) On the control of gonadotropin secretion in the rhesus monkey. *Recent Prog. Horm. Res.* **30**, 1–46.
- Lincoln, G.A. & Kay, R.N.B. (1979) Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags (*Cervus elaphus*). *J. Reprod. Fert.* **55**, 75–80.
- Lincoln, G.A. & Short, R.V. (1980) Seasonal breeding: Nature's contraceptive. *Recent Prog. Horm. Res.* **36**, 1–52.
- Lincoln, G.A., Youngson, R.W. & Short, R.V. (1970) The social and sexual behaviour of the red deer stag. *J. Reprod. Fert., Suppl.* **11**, 71–103.
- McMillin, J.M., Seal, U.S., Keenlyne, K.D., Erickson, A.W. & Jones, J.E. (1974) Annual testosterone rhythm in the adult white-tailed deer (*Odocoileus virginianus borealis*). *Endocrinology* **94**, 1034–1040.
- Mirarchi, R.E., Howland, B.E., Scanlon, P.F., Kirkpatrick, R.L. & Sanford, L.M. (1978) Seasonal variation in plasma LH, FSH, prolactin and testosterone concentrations in adult male white-tailed deer. *Can. J. Zool.* **56**, 121–127.
- Ravault, J.P. (1976) Prolactin in the ram: seasonal variations in the concentration of blood plasma from birth until three years old. *Acta endocr., Copenh.* **83**, 720–725.
- Schams, D. & Karg, H. (1969a) Radioimmunologische LH-Bestimmung im Blutserum vom Rind unter besonderer Berücksichtigung des Brunstzyklus. *Acta endocr., Copenh.* **61**, 96–103.
- Schams, D. & Karg, H. (1969b) Radioimmunologische Bestimmung von Prolaktin im Blutserum vom Rind. *Milchwissenschaft* **24**, 263–265.
- Schams, D. & Reinhardt, V. (1974) Influence of the season on plasma prolactin level in cattle from birth to maturity. *Horm. Res.* **5**, 217–226.
- Schams, D. & Schallenberger, E. (1976) Heterologous radioimmunoassay for bovine follicle-stimulating hormone and its application during the oestrous cycle in cattle. *Acta endocr., Copenh.* **81**, 461–473.
- Schams, D., Barth, D. & Karg, H. (1980) LH, FSH and progesterone concentrations in peripheral plasma of the female roe deer (*Capreolus capreolus*) during the rutting season. *J. Reprod. Fert.* **60**, 109–114.
- Sempéré, A.J. (1978) The annual cycle of plasma testosterone and territorial behavior in the roe deer. In *Environmental Endocrinology*, pp. 73–74. Eds J. Assemacher & D. Farner. Springer-Verlag, Berlin.
- Sempéré, A.J. & Boissin, J. (1981) Relationship between antler development and plasma androgen concentrations in adult roe deer (*Capreolus capreolus*). *J. Reprod. Fert.* **62**, 49–53.
- Short, R.V. & Hay, M.F. (1966) Delayed implantation in the roe deer *Capreolus capreolus*. *Symp. zool. Soc. Lond.* **15**, 173–194.
- Short, R.V. & Mann, T. (1966) The sexual cycles of a seasonally breeding mammal, the roebuck (*Capreolus capreolus*). *J. Reprod. Fert.* **12**, 337–351.
- Steinberger, A. & Steinberger, E. (1976) Secretion of an FSH-inhibiting factor by cultured sertoli cells. *Endocrinology* **99**, 918–921.
- Stieve, H. (1950) Anatomisch-biologische Untersuchungen über Fortpflanzungstätigkeit des europäischen Rehes (*Capreolus capreolus*). *Z. mikrosk.-anat. Forsch.* **55**, 427–530.
- Wandeler, A.J. (1975) *Jahrbuch des Naturhistorischen Museums der Stadt Bern* **5**, 1972–1974.
- West, N.O. & Nordan, H.C. (1976) Hormonal regulation of reproduction and the antler cycle in the male Columbian black-tailed deer (*Odocoileus hemionus columbianus*). Part I. Seasonal changes in the histology of the reproductive organs, serum testosterone, sperm production, and the antler cycle. *Can. J. Zool.* **54**, 1617–1636.
- Wislocki, G.B., Aub, J.C. & Waldo, C.M. (1947) The effects of gonadectomy and administration of testosterone propionate on the growth of antlers in male and female deer. *Endocrinology* **40**, 202–224.
- Ziegler, L. (1843) *Beobachtungen über die Brunst und den Embryo der Rehe*. Helwing'sche Hofbuchhandlung, Hannover.

Received 5 January 1982