LH, FSH and progesterone concentrations in peripheral plasma of the female roe deer (*Capreolus capreolus*) during the rutting season

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Summary. Progesterone, LH and FSH were measured in the plasma of 4 female roe deer (2 kept with the buck, 2 separated from the buck to prevent mating) from July until the end of September or October, i.e. including the rutting season from the middle of July to the middle of August. At least two distinct peaks of LH and FSH were observed before the first small progesterone increase lasting for about 5 days in late July. The next clear LH peak and, with slight individual variations, FSH peak occurred exactly when progesterone values had fallen and before they rose again in a major elevation, i.e. after ovulation. LH and, with some variations, FSH values were generally basal while progesterone was high during the rest of the study. There were no obvious differences in hormone pattern in pregnant and non-pregnant animals.

Introduction

The rutting season of the roe deer occurs from the middle of July to the middle of August. For the doe, only limited information is available about hormonal changes at that time. Plasma progesterone concentrations during gestation have been reported by Short & Hay (1966), Aitken (1974) and Sempéré (1977). More detailed information about progesterone and oestrogen concentrations in captive pregnant and non-pregnant roe deer was given by Hoffmann, Barth & Karg (1978). As far as we know no information is available about the gonadotrophin levels in blood before and after the onset of oestrus in roe deer. In the present study, therefore, serial blood samples were obtained from 4 captive does from July until October for measurement of LH, FSH and progesterone concentrations to investigate possible cyclic changes during the breeding season. The progesterone data from 2 of these does were included in a previous paper (Hoffmann *et al.*, 1978).

Materials and Methods

Experimental animals and blood sampling

Two of the animals (Does 19 and 98) were born in the wild in 1972 and trapped when 1–20 days old. After a period of bottle feeding and following the acceptance of roughage, they were housed in enclosures located in their natural environment. The other two animals (Does 2 and 61) were born in captivity in 1974. At the time of the present investigation all the does were adult. Details of housing, nutrition and management of the animals have been described previously (Barth, Giménez, Hoffmann & Karg, 1976). In Exp. A, Does 61 and 98 were separated from

the buck immediately after parturition and during the investigation to prevent mating. Blood was collected 3 times weekly from 28 June until 30 August then once a week until 19 October, 1976. In Exp. B, Hinds 19 and 2 were kept with the buck and blood was collected daily from 3 July until 25 August, then twice a week until 28 September 1978. Under the conditions of management it was not possible to observe symptoms of oestrus or mating, but Doe 19 must have conceived because 2 fawns were born in May 1979. Doe 2 failed to become pregnant although she had done so during previous years. 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 assay.

Hormone determinations

The concentrations of LH, FSH and progesterone were evaluated radioimmunologically as described by Schams & Karg (1969), Schams & Schallenberger (1976) and Hoffmann, Kyrein & Ender (1973).

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 lactoperoxidase 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 two 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 run in the assay as controls for the reproducibility of the system, was 11.5%.

FSH. A heterologous system with pure ovine FSH for labelling 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

Text-fig. 1. Binding inhibition curves of bovine LH (●) and FSH (○) compared to inhibition curves of a pituitary extract obtained from roe deer pituitaries (x).

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 two 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 as controls for the reproducibility of the system, was 13.6%. Cross-reaction and parallelism with the bovine standards were checked by inhibition curves of a pituitary extract from roe deer pituitaries; these were parallel as shown in Text-fig. 1. The pituitary material was the same as that investigated earlier by Brüggemann, Adam & Karg (1965) and was stored as a freeze-dried preparation. In both assays (LH and FSH) 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

Text-fig. 2. Concentrations of FSH, LH and progesterone in peripheral plasma of 4 does before, during the rutting season and the next 6–10 weeks.

double-antibody method. A precipitating antiserum against rabbit or guinea-pig gamma-globulins respectively was added after an additional 48–72 h incubation. Separation of bound from free labelled hormone was performed 24 h later. Bovine pituitary preparations were used as the reference standards for FSH (NIH-FSH-B1, biological activity 0.49 times NIH-FSH-S1) and for bovine LH-DSA (prepared in our laboratory, biological activity of $1.0 \times$ NIH-LH-S1).

Progesterone. Endogenous oestrogens, androgens and corticoids exhibited no cross-reactivity with the antiserum (raised in rabbit against 11α -hydroxyprogesterone hemisuccinate-BSA) but there was a 7.5% cross-reaction with desoxycorticosterone. However, the specificity was further ensured by an extraction step with petroleum ether. The reagent blank was <10 pg/ml. Recovery studies with labelled progesterone added to cow and roe deer plasma gave similar results in the range between 79 and 82%. Dilution curves of roe deer plasma were parallel to the progesterone standard curve. The intra-assay CV calculated for two different plasma pools was on average 7.2%. The interassay variation, expressed as the mean of 3 different roe deer samples run in the assay as controls, was on average 11%. Sensitivity was 30 pg/ml.

Results

The hormone values obtained from the 4 females are shown in Text-fig. 2. Progesterone concentrations remained low (with small fluctuations) until the middle or end (Doe 98) of July when increased progesterone concentrations (up to 0.9 ng/ml) were observed for about 5 days. The decrease was followed by a major increase with continuously high plasma progesterone concentrations throughout the period examined. In Doe 98 the curve was smooth but the values for the other animals fluctuated markedly. In 3 of the animals (Does 98, 2 and 19) at least two distinct LH peaks were observed before the first small increase of progesterone values occurred. In Doe 61 (with a lower sample frequency) there was only one distinct peak but two smaller elevations followed. In all 4 females there was a distinct LH peak after the first increase of progesterone. This peak, lasting some days, occurred when the progesterone values were low before next becoming elevated. The FSH patterns in most cases were similar to those obtained for LH. There were distinct elevations of FSH at the start of the experiment in 3 females, but they were not related to changes in LH. After the second increase of progesterone values of LH, and in general FSH, remained constant at baseline concentrations.

Discussion

The remarkable fluctuations of gonadotrophin levels at the start of this experiment indicate the activity of the hypothalamic-hypophyseal system even before the onset of the rutting season. The first distinct progesterone elevation lasted for about 5 days in all 4 animals and was within the season. However, even if this was preceded by distinct peaks of both gonadotrophins we cannot say from our data whether this progesterone was from luteinization of a follicle with or without ovulation. Of the ensuing increases of gonadotrophin concentrations, that for LH was clearly the last one and was obviously associated with an ovulation. This was followed by the formation of a normal corpus luteum which remained throughout the period of study whether mating and pregnancy occurred or not. Hoffmann *et al.* (1978) have shown that even in non-pregnant females progesterone levels indicating functional luteal tissue are measurable until March/April although a decline starts in February. This is in agreement with the morphological observations of Aitken *et al.* (1973) who reported luteal regression in non-pregnant does after January or February. It therefore seems likely that this spontaneous 'pseudopregnancy'

expresses an autonomous mechanism for pregnancy protection in a species in which the conceptus is perhaps a weak regulator of its own security during the period of delayed implantation.

The hormone profile characterizing 'the build-up' to the breeding season in the roe deer resembles the pattern which has been found in domestic species to be associated with the start of cyclicity and reflecting in general the maturation processes of the hypothalamus-pituitary-ovary axis. In seasonally breeding sheep, similar profiles were observed at the end of the period of anoestrus, i.e. small increases of progesterone (Thorburn, Bassett & Smith, 1969; Walton, Cunningham, Temple & Bowman, 1974) and fluctuations in the LH levels (Yuthasastrakosol, Palmer & Howland, 1975). In cattle before puberty (Schams & Butz, 1972; Gonzales-Padilla, Wiltbank & Niswender, 1975) and before the resumption of oestrous cycles after parturition (Lamming, 1978; Schams *et al.*, 1978), there is usually an early small increase of progesterone lasting about 5–9 days preceded by fluctuations of LH and FSH levels.

The lack of correlation between FSH and LH peaks at some times, e.g. at the start of sampling in Does 2 and 98 and after the 'ovulatory surge' when there are still a few minor FSH fluctuations although LH has already reached baseline concentrations, is not surprising. Narayana & Dobson (1979) demonstrated by means of administration of antibodies against GnRH in sheep that GnRH controls the preovulatory synchronous LH and FSH surges, but not the second FSH surge. During the oestrous cycle of cattle a wave-like pattern can be observed for FSH values but there is no correlation with LH levels (Schams, Schallenberger, Hoffmann & Karg, 1977).

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