Seasonal reproductive endocrine profiles in two wild mammals: the red fox (Vulpes vulpes L.) and the European badger (Meles meles L.) considered as short-day mammals

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Abstract. The annual cycle of the testicular function (testis and epididymis weights and plasma testosterone levels) were considered in relation to seasonal variations in plasma LH and prolactin concentrations in two wild European mammals: the badger and the fox. Phase relationships were established between the annual prolactin cycles and daylight duration. The influence of castration on the seasonal variations in plasma LH levels was also studied. The resumption of activity in the testicular function occurs during autumn for both species. The reproduction period begins in winter but it is over by the beginning of spring for the fox whereas for the badger it lasts until early summer. In the same way, the annual cycle of the gonadotrophic function which, in the fox, presents only one maximum at the end of autumn, is bimodal in the badger with one maximum in January and a second in June. On the other hand, both species have a similar annual prolactin cycle, which shows an increase from the winter solstice onwards, in synchronization with the increase in daily light duration. The highest prolactin levels are measured in spring followed by a decrease during summer. This result calls into question the role played by prolactin in the regulation of the testicular function in as far as the two species have an annual reproductive cycle of the 'short-day' type (onset of activity occurring before the winter solstice) but show seasonal prolactin variations similar to those described for 'long-day' species.

Considering the annual cycle of the gonadal function in relation to the seasonal variations in natural photoperiod, the length of the daylight period plays an essential role in the control of the annual reproduction cycle (for review, see Benoit & Assenmacher 1970). Two types of species can be distinguished: long-day species (roe deer, ferret, marten, stone-marten) whose annual cycle of testicular activity is characterized by a winter recrudescence when daylight is increasing and short-day species whose gonadal activity increases after the summer solstice (white-tailed deer, red deer) or autumn equinox (mink).

In the first case, mating takes place in late winter or in spring except for the roe deer, late July to early August, whereas in the second case it occurs in late summer or during autumn. Thus it seemed interesting to study the annual cycle of the testicular function in two mammals, the fox and badger, whose main reproductive phase (rutting and mating) begins very early in winter, in January (Maurel & Boissin 1981). In the badger, there is also a second phase during which high testosterone levels can be measured until June or July (Maurel et al. 1977). To enable us to situate with precision the onset of the gonadal function, we compared the seasonal cycle of plasma concentration in LH and prolactin to the cycle of testicular weights and plasma testosterone concentration in as far as natural photoperiod can exercise regulatory action only through the gonadotrophic hormones. Many studies on domestic mammals have shown that direct relationships exist between the seasonal fluctuations in plasma LH and plasma testosterone concentration but similar studies on wild animals are rarer except studies on cervidae (white-tailed
deer: Mirarchi et al. 1978; red deer: Lincoln & Kay 1979; roe deer: Sempéré & Lacroix 1982; Schams & Barth 1982) or on the mongoose (Soares & Hoffman 1981) where synchronization between plasma gonadotrophic and testicular hormone levels is shown.

The importance of prolactin in the regulation of the annual cycle in the testicular function is still a subject of controversy in spite of the fact that it has often been considered that this hormone has an antigonadotrophic effect (Bohnert et al. 1976; Beck et al. 1977). However, it is known that in domestic mammals prolactin presents a clearly defined annual cycle: sheep (Pelletier 1973; Ravault 1976), cattle (Schams & Reinhard 1974; Lacroix et al. 1977), goats (Buttle 1974; Muduuli et al. 1979). The study of the annual endocrine cycle in wild animals can be an important source of information on the role played by prolactin in the regulation of testicular activity because of the considerable variations in seasonal rhythms and the phase relationships that can be established.

Materials and Methods

This study was carried out in the Chièze Forest (midwest France, latitude: 46°07’N; longitude: 0°25’W).

Animals

The adult male badgers and foxes were trapped in the forest near the laboratory and after arrival they were housed individually in outdoor pens in their natural environmental conditions. The animals were fed daily (badgers: one-day-old chicks; foxes: hen carcasses); water was provided ad libitum. Adult animals were distinguished from immature by the size of the baculum, the cranial suture, the teeth aspect.

Longitudinal hormone profiles

The number of badgers and foxes is shown in Table 1. The badgers and foxes were castrated during the period of testicular activity. Monthly or bimonthly, blood samples were taken from the radial vein after a slight anaesthesia with diethylether between 9 and 11 a.m.

Assay procedure

Testosterone assay. Plasma testosterone levels were measured by a radioimmunoassay method without chromatography described by Maurel et al. (1981). Lowest detectable levels were 8 pg per tube; the coefficients of intra-assay and inter-assay were respectively 4.2 and 9.9%.

LH assay. A double antibody radioimmunoassay was developed for determining plasma LH in the fox and the badger. Two homologous ovine-ovine and rat-rat R.I.A. systems were used as they were seen to cross-react well with the plasma LH in the fox and the badger respectively. The following highly purified pituitary LH were

Table 1.

Plasma LH levels in intact and castrated animals during the annual cycle. The number of animals studied is indicated in parentheses.

<table>
<thead>
<tr>
<th>Months</th>
<th>Fox</th>
<th>Badger</th>
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<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Castrated</td>
</tr>
<tr>
<td>June</td>
<td>1.09 ± 0.06 (8)</td>
<td>9.9 ± 4.3 (6)</td>
</tr>
<tr>
<td>July</td>
<td>1.15 ± 0.07 (10)</td>
<td>3.9 ± 0.6 (6)</td>
</tr>
<tr>
<td>August</td>
<td>1.7 ± 0.7 (7)</td>
<td>1.7 ± 0.7 (7)</td>
</tr>
<tr>
<td>September</td>
<td>1.12 ± 0.07 (4)</td>
<td>10.5 ± 1.3 (6) *</td>
</tr>
<tr>
<td>October</td>
<td>1.24 ± 0.15 (7)</td>
<td>9.4 ± 1.1 (6) *</td>
</tr>
<tr>
<td>November</td>
<td>3.02 ± 0.51 (8)</td>
<td>9.8 ± 1.2 (6) *</td>
</tr>
<tr>
<td>December</td>
<td>2.19 ± 0.26 (7)</td>
<td>6.3 ± 1.6 (10) *</td>
</tr>
<tr>
<td>January</td>
<td>2.14 ± 0.35 (6)</td>
<td>9.4 ± 0.9 (10) *</td>
</tr>
<tr>
<td>February</td>
<td>2.50 ± 0.35 (11)</td>
<td>10.5 ± 1.6 (9) *</td>
</tr>
<tr>
<td>March</td>
<td>1.71 ± 0.22 (10)</td>
<td>9.0 ± 3.7 (10) *</td>
</tr>
<tr>
<td>April</td>
<td>1.28 ± 0.13 (19)</td>
<td>7.5 ± 0.9 (6) *</td>
</tr>
<tr>
<td>May</td>
<td>1.21 ± 0.16 (13)</td>
<td>8.1 ± 1.1 (6) *</td>
</tr>
</tbody>
</table>

Statistical analysis: * P < 0.01; NS: non-significant.
used: Ovine luteinizing hormone: oLH-M3 = 1.8 × LH-NIH-S1 used for iodination and standard curve; oLH-M1 = 1.5 × LH-NIH-S1 used for antisera preparation. Rat luteinizing hormone: rLH-Sx-1-1 = 1.6 × LH-NIH-S11 used for antisera preparation, iodination and standard curve.

The anti-ovine LH serum was raised from guinea pigs and the anti-rat LH serum from rabbits. The anti-γ-globulin serum from the respective species was used as second antibody. The specificity of the antisera was checked (Pelletier et al. 1968).

Rat and ovine pituitary LH preparations were similarly labelled with radioactive iodine (125I, Amersham, IMS 300). The labelling was performed by Greenwood and Hunter’s Chloramine T method modified so as to obtain a highly purified labelled hormone with a low specific activity (80 μCi/μg). The immunological activity of the labelled hormone was controlled by reaction with excess LH antibody.

All reagents were diluted in 0.025 M HSA barbitone buffer, pH 8.6. The present methodology, applied for fox and badger plasma respects the conditions already established for ovine and rat homologous assay respectively in the following way:

Labelled antigen: ovine LH (oLH-M3) was used for the fox, and rat LH (rLH-Sx-1-1) for the badger. The purified [125I]LH was diluted so as to obtain approximately 5000 and 10 000 CPM per assay tube for ovine and rat LH respectively.

Unlabelled antigen (standard and plasma samples): the purified LH preparation was further diluted and used for the standard curve preparation in the homologous assays described. Volume of standard and plasma sample were 100 μl for 500 μl of total react volume.

Anti LH serum: the antisera were used at a final dilution of 1:400 000 (anti ovine LH serum) and 1:70 000 (anti-rat LH serum). One per cent of normal serum was added to this which served as carrier serum for immunoprecipitation.

The tubes were incubated for 4 days at 4°C. Then the second antibody was added and incubated for 24 h at 4°C. The precipitate was rinsed and all tubes centrifuged for 45 min at 4200 r.p.m. at 20°C.

The supernatants were decanted and the bound [125I]LH complex was counted in a gamma spectrophotometer (Intertechnique CG 30). The LH concentrations in plasma samples were estimated from the calibration curve and expressed in ng per ml of standard LH.

The validation of the assay demonstrated a precision of 8.6 and 12.9% (C.V. inter-assay for fox and badger respectively) and of 2.8 and 8.7% (C.V. intra-assay for fox and badger respectively). The sensitivity of the method was 0.3 and 0.6 ng/ml of standard reference for fox and badger respectively. The specificity was verified by the parallelism test of serial plasma dilutions with standard curve in the two species: after logit-log transformation, the standard curve was compared with the plasma dilution curve in the two species. The slope was 3.08 ± 0.12 and 2.42 ± 0.16 for the standard curve and 2.72 ± 0.32 and 2.15 ± 0.27 for the plasma dilution curve for fox and badger respectively. The difference between the standard and plasma samples for each

![Graph](https://via.placeholder.com/150)

**Fig. 1.**

Plasma LH release in fox and badger after a single iv injection (→) of LRH. (2 μg/kg body weight).
Seasonal variations in testis and epididymis weights in the fox and the badger.

The number of animals is given for each month.

species was not significant (Student's t-test). When four groups were studied (variance analysis) the same result was obtained.

The analysis of the plasma LH release pattern after LRH treatment and after castration were used as biological tests to check the cross-reaction specificity of the assay system concerning the other plasma glycoprotein hormones.

The LH release after LRH injection (2 µg/kg body weight) is shown in Fig. 1. A significant increase of the plasma LH level was observed as early 5 min after injection from 1.77 ± 0.12 to 7.70 ± 0.77 ng/ml of standard oLH in foxes (P < 0.001) and from 3.29 ± 0.22 to 8.21 ± 2.05 ng/ml of standard rLH in badgers (P < 0.001).

In castrated foxes the mean plasma LH level was significantly higher than in the intact animals during the breeding and non-breeding season (440 and 672% respectively; P < 0.001). In castrated badgers a significantly higher plasma LH levels were recorded during the non-breeding season only (471%; P < 0.001).

Prolactin assay. The fox and badger plasma prolactin was measured in the same heterologous radioimmunoassay porcine/ovine according to the method of Kann (1971).
The purified pituitary prolactin (pig, SP 162 C) and the prolactin standard (sheep, Prl NIH-P-S6) were provided by N.I.H. Baltimore (USA). The antibody antiovine Prl was obtained after guinea pigs immunization with SP 6 standard. Assay sensitivity was approximately 1.0 ng/ml. In our assay conditions, the inter- and intra-assay coefficient were estimated 15 and 8% respectively.

The mean values of hormonal concentrations have been shown with the standard error; variance analysis (test F) was used for statistical exploitation of the results (comparison of the mean values).

Results

Seasonal variations profile of the testicular and epididymis weights and of the plasma testosterone
Seasonal variations in testicular weight (Fig. 2) were considerably greater in the fox than in the badger (max/min ratio: fox = 6, badger = 3). In both species, testicular weight increase occurs during late autumn. In the fox, testis weight decreases sharply immediately after the reproduction period (March

![Graphs showing seasonal variations in plasma testosterone, LH, and prolactin concentrations in relation to the daylength, in the adult male badger and fox.](image-url)
vs April: $P < 0.01$) whereas in the badger it remains high until summer. The epididymal ponderal variations are similar to the variations in testicular weight in the two species. Examination of the curve showing seasonal cycles in plasma testosterone concentration confirms the short duration of the period of endocrine testicular activity in the fox and the longer period lasting several months (from January – February to July) in the badger (Fig. 3).

Annual prolactin cycle (Fig. 3)

In the badger, prolactin which is at its lowest level in autumn (between 2.0 and 2.7 ng/ml) increases during the months of December and January and reaches a maximum in April (11.7 ± 1.0 ng/ml). Level remains high until July – August (10.2 ± 0.7 ng/ml) then decreases significantly during September – October. In the fox, the lowest levels occur once again in autumn (September – November: 3.03 ± 0.32 ng/ml) and the highest are measured at the beginning of spring (May: 15.6 ± 1.3 ng/ml). On the other hand, the amplitude of seasonal variations is greater in the fox than in the badger.

Annual cycle of plasma LH concentration in intact and castrated animals (Fig. 3, Table 1)

In the badger, the LH cycle is characterized by a bimodal aspect with a first maximum level in January (11.7 ± 3.9 ng/ml) and a second at the end of spring (June: 9.9 ± 0.5 ng/ml). The sharp decrease in the plasma LH concentration observed from March to May is statistically significant (February or June vs March, April or May: $P < 0.01$). The lowest values are observed only during summer and the onset of the LH release occurs in autumn, late in November (August or September vs November: $P < 0.01$).

The annual variations in plasma LH concentrations in the castrated badger are roughly identical to the annual cycle described in the animal intact. The highest LH levels are measured in early winter and in late spring. However, the variations observed are no longer statistically significant. There is no difference between the annual mean levels in LH plasma in the intact animal (5.4 ± 0.8 ng/ml) and the castrated animal (7.7 ± 0.7 ng/ml). However, during the period of sexual inactivity, plasma LH level measured in the intact animal (August – November: 1.9 ± 0.2 ng/ml) are significantly lower than for the castrated animal (6.3 ± 0.8 ng/ml; $P < 0.01$).

The annual cycle in plasma LH concentration in the fox shows only one maximum level. The LH increase occurs in November and remains important until March; during this period, the mean level (2.37 ± 0.35 ng/ml) is significantly higher than at any other time during the year (mean level April – September: 1.19 ± 0.07 ng/ml). Like the badger, the LH increase in autumn occurs 2 months before the increase in testosterone.

In the fox, castration, even long-term castration, brings about a considerable increase in the LH plasma levels (mean level in castrated animals: 9.06 ± 0.46 ng/ml vs mean level in normal animals: 1.86 ± 0.12 ng/ml); this high level is statistically significant all year round and no seasonal variation is observed.

Discussion

The study of the different parameters which were chosen to characterize the annual cycle of the testicular function shows that in both species recrudescence of activity occurs during autumn after spring and summer inactivity in the fox but after only summer inactivity in the badger. Thus, although the maximum of testicular activity is observed after the winter solstice when daylight is increasing, the activation of the hypophyso-gonadal axis takes place before the winter solstice, in inverse conditions of natural photoperiod, as is shown by the analysis of the seasonal variations in plasma LH concentration: LH increases in November when daylight is always decreasing and daily light duration is shorter than that of the period of darkness.

Because of the situation of the onset of LH release phase in relation to the seasonal variation in natural photoperiod, the badger and the fox can be considered as short-day mammals. However, in this respect, their situation is not classical since it is generally admitted that in short-day mammals the annual recrudescence begins after the summer solstice when daily light duration is decreasing even though daylight is still longer than darkness (white-tailed deer: Mirarchi et al. 1978; pygmy goat: Muduufi et al. 1979; red deer: Lincoln & Kay 1979; ram: Schanbacher & Lunstra 1976). It must be noted, however, that recent studies on the mongoose (Soares & Hoffman 1981) show that recrudescence of testicular activity (testis weight, gonadotrophic level) although occurring in late autumn takes place before the winter solstice.
In wild mammals such as the badger and the fox it is difficult to establish experimentally the causality of the phase relationships between the seasonal variations in natural photoperiod and in testicular activity. However, the comparison of our results with those obtained in another carnivore, the mink, which like the fox and the badger, resumes gonadal activity in autumn (Boissin-Agasse & Boissin 1979) leads us to think that the decrease in daylight duration from the autumn equinox to winter solstice is responsible for the onset of testicular activity; it has been shown experimentally in the mink (Boissin-Agasse et al. 1982) that the recrudescence of the testicular function can only take place when daylight duration is less than 12 h.

In our latitude, when the increase in plasma LH concentration is observed, in the fox and the badger, at the end of October, daily light duration is roughly equal to 10 h. We can put forward the hypothesis that the decrease in daily light duration between autumn equinox and winter solstice is responsible for the resumption of gonadotrophic activation in the fox and the badger. Starting from the end of the winter reproduction period, the plasma LH concentration decreases sharply in the fox and remains at a very low level during the other months of the year whereas in the badger there is a second period of activity in the gonadotrophic function at the end of spring, in relation to the second reproduction period (Maurel 1981).

To explain the sharp decrease in gonadotrophic activity at the end of winter, in both the fox and the badger, it can be considered that this decrease is related to the increase in daylight length which might inhibit the neuroendocrine structures controlling gonadotrophic pituitary activity as has been shown in the mink, which presents a photoperiodic response of the 'short-day' type (Boissin-Agasse et al. 1982). This hypothesis might explain the functioning of testicular activity in terms of annual cycle in the fox; however, it cannot explain the onset of gonadotrophic activity observed in the badger at the end of spring. In the latter case, two hypothesis can be suggested. Firstly, the high levels of testosterone measured during the month of February might inhibit the gonadotrophic function which would lead to low plasma concentration in LH from March to May. The second recrudescence would then be due to the removal of inhibition (exercised by testosterone on the neuroendocrine structures) owing to a decrease in androgen levels. This decrease in plasma testosterone levels is related to an increase in metabolic clearance rate of the testosterone (Maurel & Boissin 1982) which at this time of the year accounts for an increase in peripheral use of the male hormone. This might be provoked by the rapid increase in plasma thyroxine concentration also observed at this time of the year (Maurel et al. 1977) for it has been shown that the thyroid hormone stimulates the peripheral androgen metabolism (Assenmacher et al. 1975). According to the other hypothesis, the second recrudescence of the gonadotrophic function is evidence of the existence of a 'circasemestrial' activity on the level of the nervous and neuroendocrine structures responsible for the androgenic cyclicity of the reproduction cycle (Assenmacher 1974). In this case, the second recrudescence would be independent of any natural photoperiod influence. Because of the similarities observed in the LH cycles of the normal and the castrated badgers, the hypothesis involving the negative hormonal feedback in the determination of both the winter decrease and the spring recrudescence of gonadotrophic activity cannot be taken into consideration; similarly, it is no longer possible to discuss such a cycle in terms of testo-thyroid negative interactions. However, the absence of statistical significance characterizes the profile of the variations in plasma LH concentration in the castrated animal, because of the importance of the dispersion of the measured values in January and in July but this only emphasizes the intense pulsatile gonadotrophic activity during these two periods of the year. A similar type of phenomenon might also be true for the castrated fox, which would then mask the cyclic nature of LH plasma variations. It must be noted in the latter species that the inhibiting action of testosterone on the gonadotrophic function is particularly important as is shown by the increase in LH plasma level in the castrated animal.

The seasonal variations in prolactin in the fox and in the badger are similar to those described for other domestic (see Introduction) or wild mammals (white-tailed deer: Mirarchi et al. 1978; wild boar: Mauger 1982; roe deer: Schams & Barth 1982; Sempéré & Boissin 1983) in which the annual cycle of plasma prolactin concentration shows direct phase relationships with seasonal variations in natural photoperiod. In some species (sheep, goat, white-tailed deer), the recrudescence of testicular activity occurs after the summer solstice when prolactin plasma concentration begins to decrease, whereas in the roe deer, on the contrary, the
resumption of gonadal activity is concomitant with the increase in plasma prolactin. Finally, in the fox and the badger, the LH levels increase when prolactin is at its lowest level; on the other hand, the increase in plasma prolactin concentration after the winter solstice does not inhibit gonadotrophic activity. Thus the results obtained in the fox, the badger and in the roe deer (Sempère & Boissin, 1983) tend to call in question the antagonadotrophic role attributed to prolactin on the basis of studies carried out on the rat (Beck et al. 1977) or observations on ungulates presenting a 'short-day' photoperiod response.

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References


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