Changes in plasma testosterone, thyroxine and triiodothyronine in relation to sperm production and remex moult in domestic ganders

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Summary — Changes in plasma testosterone (T), thyroxine (T4), triiodothyronine (T3), semen output and remex moult were studied in domestic ganders. A bimodal pattern in both plasma T and sperm concentration was observed during the annual cycle. Ganders started to produce semen at the end of January; maximum semen volume (0.32 ± 0.04 ml) and sperm concentration (148 ± 38 x 10⁶/mm³) were reached in March and a marked decrease was observed after mid-April, when the moult of the remiges began. Plasma T3 levels peaked in February (9.7 ± 0.6 nmol·l⁻¹) and this peak coincided with maximum T concentrations (9.8–10.4 nmol·l⁻¹). Elevated levels of T4 were found from late February until mid-April (31.0–33.6 nmol·l⁻¹). Plasma T concentration was low at all stages of remex moult and regrowth. Decreased T4 levels were found in ganders during remex regrowth from the “brush” to half of the full primary growth stage. Higher plasma T4 levels were found before and after this stage of the moult. A reverse pattern was observed for T3 concentrations.

testosterone / thyroxine / sperm concentration / semen volume / moult / gander

Résumé — Testostérone, thyroxine et triiodothyronine plasmatiques en relation avec la production spermatique et la mue chez le jars domestique. Les changements de concentration plasmatique de testostérone (T), de thyroxine (T4) et de triiodothyronine (T3) ainsi que la production de spermatozoïdes et la mue des rémiges ont été étudiés chez le jars domestique. La sécrétion de T et la concentration des éjaculats en spermatozoïdes présente un profil bimodal au cours de l'année. Les premiers spermatozoïdes sont observés à la fin du mois de janvier, leur production est maximale en mars (57 ± 17 x 10⁶/éjaculat) puis diminue en avril au moment où les rémiges commencent à tomber. La concentration de T3 est maximale (9,7 ± 0,6 nmol/l) en même temps que celle de T (9,8 ± 10,4 nmol/l). La concentration en T4 augmente de la fin février à la mi-avril (31–34 nmol/l). Les concentrations plasmatiques de T sont faibles pendant la mue des rémiges; celles de T4 passent par un minimum aux stades 4–5 de la mue et présentent un profil inverse de celui des concentrations plasmatiques de T3.

testostérone / thyroxine / concentration spermatique / mue / jars

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INTRODUCTION

The domestic goose shows the seasonal pattern of reproductive activity which its wild ancestor, the Graylag goose (Anser anser) evolved in adapting to seasonal changes of environmental conditions. This reproductive strategy is convenient in the traditional extensive breeding conditions characterized by natural incubation of eggs and parental care. However, the introduction of large-scale goose production requires the artificial incubation of eggs and a substantial increase of reproductive performance, particularly an extension of the reproductive period in the annual cycle. Endocrine mechanisms control the development of reproductive capacity, and their study seems to be important in improving the reproductive performance in this species of waterfowl.

In many avian species of mid and high latitudes, day length is the major environmental trigger in the initiation and synchronization of annual reproductive cycles. Increasing day length in the spring induces acceleration of gonadotropin secretion, and this precedes and stimulates vernal increase in the gonadal activity of birds (Farner and Gwinner, 1980; Farner and Wingfield, 1980). Reproductive activity is terminated by the development of long-day refractoriness which results in an inhibition of gonadotropin-releasing hormone synthesis and gonadal regression under long photoperiod (Nicholls et al, 1988). The mechanisms involved in the development of long day refractoriness are still unclear but the role of interactions between seasonally elevated sex hormones and thyroid hormones, and between gonadotropins and prolactin have been suggested (Assenmacher and Jallageas, 1980; Follett and Nicholls, 1984; Nicholls et al, 1988; Dawson, 1989). Geese are the only species of poultry regularly used in breeding for several seasons. A study of endocrine control of seasonal breeding could improve their management and the benefits of their breeding. However, such studies are rare in geese (Košutzký et al, 1982; Lazar, 1983; Péczely et al, 1985; Zeman et al, 1987) and most data about the endocrine control of reproduction in waterfowl come from studies on ducks (Jallageas and Assenmacher, 1974; Paulke and Haase, 1978). The objective of this study was to establish the relationship between changes in semen production and plasma testosterone concentration and to evaluate the relationships among semen output, primary remex moult and plasma concentrations of testosterone (T), thyroxine (T4) and triiodothyronine (T3) in ganders.

MATERIALS AND METHODS

Ganders of a parent line of broiler breeder geese IJages were used. They were hatched in March and studied in the following year, ie in both trials during their first reproductive season. The birds were housed in groups of 10 in pens with a slatted floor (2.5 x 2 m) and an open concrete yard (2 x 3 m) supplied with a trough containing running water (2 x 1.5 m). A complete goose feed (18.2% crude protein and 10.46 MJ metabolizable energy/kg) was provided.

Experiment I

Thirty ganders were exposed from 15 October to 20 December to a short photoperiod of 8 h of light and 16 h of darkness (LD 8:16) and restricted feed intake (180 g of feed per gander per d). From 20 December the feed was provided ad libitum and day length was increased gradually by artificial light (incandescent 100 W bulbs providing a light intensity of 10–50 lx at the level of the birds' heads) to a regime of LD 12:12 at the end of January. This light-dark cycle was held until 21 March and then the ganders were exposed to naturally increasing day length (47° 18' N, 17° 13' E).
Blood and semen samples were taken at monthly intervals in the first 10 days of each month at the same time of day (between 8.00–10.00 h). The semen was collected by dorso-abdominal massage. Semen volume was determined indirectly (1 ml = 1 g) by weighing (Williams and de Reviers, 1981) and sperm concentration by a haemocytometer. Blood was taken from the wing vein immediately (within 1 min) after semen collection because this treatment did not significantly affect plasma testosterone concentration (Zeman, unpublished results). Heparinized blood was centrifuged at 2 500 g for 10 min; the plasma was removed and stored at -20 °C until assayed for testosterone.

**Experiment II**

Another 30 ganders were kept during the next year under the same management conditions as trial I, only the short photoperiod LD 8:16 lasted from 15 November to 10 January. Blood and semen were collected more frequently (2–3 weeks) during the reproductive season (January–June) in the same manner as in Experiment I. The semen volume and sperm concentration were determined.

The concentrations of hormones were measured by radioimmunoassay. Thyroxine and triiodothyronine were estimated from unextracted plasma using commercial RIA-kits (Institute of Radioecology and Nuclear Technology Application, Košice, Czechoslovakia). Testosterone was determined by direct RIA with 125I tracer (Zeman et al., 1986). Standards in all 3 methods were made up in gander's plasma from which endogenous steroids and thyroid hormones were removed (Abraham, 1977).

At the end of the reproductive season the moult and the replacement of primary remiges were recorded according to Herremans (1986). A 10-step scale was used for the determination of the stages of remex moult in which 0 = old (1 week before a drop), 1 = dropped, 2 = pin, 3 = brush, and 4–10 = tenths of the full-grown length. Under this system, each bird was individually examined at 2–3-d intervals and a moult score of 1–10 was given for the 1st, 5th and 10th primaries. The global score was a mean from these 3 primaries. In most cases all primaries were in the same stage of moult, as is usual in Anseriformes.

Experimental values were analyzed by 2-way analysis of variance as a randomized complete block design (Sokal and Rohlf, 1969) with the ages at the respective stages of feather moult as the fixed treatment effects and the individuals constituting the randomly chosen blocks. Levels of significance were determined by Duncan's multiple range test.

**RESULTS**

**Experiment I**

A bimodal pattern of plasma testosterone concentration was found in ganders submitted to a modified light regime during the annual cycle (fig 1). High plasma T levels were determined in December and January. Thereafter the concentration decreased dramatically and low levels were found from March to July. The second an-
nual peak of concentration of this androgen was observed during August and September, while low levels were recorded in October and November.

Sperm concentration also showed a bimodal pattern during the annual cycle of ganders. Spermatozoa were found at first in January and maximum sperm concentration was determined at the beginning of April. After this peak, sperm concentration decreased and no spermatozoa were found in July and August. A second, autumnal, reproductive period lasted from September to November and the peak of sperm concentration was found in October. The changes in testosterone levels preceded those in sperm production by about 2–3 months.

**Experiment II**

Concentrations of plasma T were low in December and maximum concentrations were found in January and mid-February (fig 2). Thereafter, concentrations decreased \( (P < 0.05) \) and minimal levels were found in mid-May. The increase in plasma T was followed by a rise in plasma \( T_3 \) levels (fig 2) with maximum values at the beginning of February. Plasma \( T_4 \) increased from February to mid-March and was high until May. The decrease of plasma testosterone concentration and the increase in the \( T_4 \) levels preceded a drop in semen quality and primary moult. The marked decline in semen volume and sperm concentration was accompanied by the onset of primary moult in a group of ganders (fig 3). Individual birds ceased to produce semen a week before the primaries were dropped. The sequence of remex moult was ascendant (from the apex of the wing towards the body) and the primaries dropped from both wings almost simultaneously in 1–2 d, even though some variability in the temporal pattern of remex moult was observed. The moult and replacement of remiges lasted approximately 6–7 wk. During this period the ganders did not produce spermatozoa and their copulatory organs had regressed. The moult of contour-feathers occurred earlier than that of the primaries; however, it was not evaluated in detail.

Fig 2. Changes of plasma \( T, T_3 \) and \( T_4 \) in ganders; \( M \pm SEM, n = 30. \) Photoperiod LD 8:16 was applied from 15 November to 10 January. Subsequent regime was the same as in experiment I.

Fig 3. Changes in semen volume, sperm concentration and % of moulting ganders. Values of sperm are \( M \pm SEM, n = 30. \)
A more precise relationship between hormone levels and primary moult was found when the hormone concentrations (from periodical samplings) were arranged according to the actual stages of the primary moult and regrowth (fig 4). Plasma testosterone concentrations were low in all sets (< 3 nmol/l), ie from ganders 1 week before the primary moult to ganders with fully-renewed primaries with no significant differences among these groups. Initial stages of primary replacement (stages 3–7) were characterized by decreased plasma T4 levels, but these increased again to initial values during the final stages of primary growth. Low plasma T3 levels were found in ganders at the beginning and at the termination of primary moult. Significantly (P < 0.01) increased T3 levels were measured in stages 3–7 of primary regrowth.

**DISCUSSION**

The absolute levels and biphasic pattern of plasma testosterone concentrations found during the annual cycle in ganders correspond with our previous results (Košutzký et al, 1982, 1984) and with those of Péczely et al (1985). These findings suggest the existence of an inherent biphasic testicular cycle profile in ganders, as already found in extensive studies of hormonal changes during the sexual cycle in drakes (Jallageas and Assenmacher, 1974; Balthazart and Hendrick, 1976; Paulke and Haase, 1978).

The peak in plasma T concentration in experiment I was already determined in December, ie before the photoperiod was artificially increased. Moreover, high testis weight was found in ganders kept under...
the same lighting regime in January (Ze-
man et al, 1987). Both these findings sug-
gest that the photoperiod LD 8:16 was suf-
ficient for the development of gonads at
this phase of the gander's annual cycle, as
found in some wild birds (Gwinner and
Gänshirt, 1982; Sharp et al, 1986b). Owing
to less frequent blood sampling it is im-
possible to determine whether the T peak
in experiment II occurred before or after
the beginning of the day length increase.
Nevertheless, it was not found exactly at
the same time of year as in experiment I.
We suppose that sexual development is
initiated after termination of photorefractor-
iness. Applied photoperiod and other envi-
ronmental conditions together with the age
of the birds, their previous life history and
nutrition determine the rate of subsequent
sexual development as well as the onset
and duration of the reproductive period.
Therefore, a variation in these factors may
account for differences in the exact timing
of the reproductive cycle in different years.
On the other hand, a modification of envi-
ronmental conditions and nutrition may be
a way of controlling the temporal pattern of
the annual reproductive cycle in goose
farming.

The second peak in plasma T is accom-
panied by the second, autumnal, reproduc-
tive period in the annual reproductive cycle
of ganders and the second egg-laying peri-
d in geese (Grom, 1971; Lazar, 1983);
this is not observed in wild birds. The an-
nual profile of testosterone differs partly
from that found under a natural photoperi-
od (Košutzký et al, 1982; Péczely et al,
1985) by its peak amplitude and duration
of high plasma T levels. One of the rea-
sons for a relatively short duration of high
peak levels could be the fact that the gan-
ders were kept without females. High T
levels in males during the egg-laying stage
are stimulated by sexual behavior of fe-
male s in some species of passerines
(Moore, 1982; Wingfield et al, 1989) and
also in ganders (Rosinski, 1989; personal
communication).

Semen volume and sperm concen-
tration were found to be highly variable and
their range corresponded with our previous
results (Zeman et al, 1985). There was a
phase-shift between annual rhythms in se-
men output and plasma T concentration.
Maximum sperm output was preceded by
maximum plasma T levels in both vernal
and autummal reproductive periods. During
vernal reproductive season culmination
(March–April), plasma T levels represented
only 30–40% of the peak values deter-
mined in January and February. Mean tes-
tis weight in April represented approxi-
mately 50% and in May 20% of the
maximum testis weight found in February
(Zeman et al, 1987). These results show
that in spite of the decrease in plasma T
levels, viable spermatozoa were produced
by ganders for 3–4 months afterwards.
The high plasma T levels seem to corre-
spond to behavioral activities occurring at
the beginning of the reproductive period in
water-fowl (aggressive behavior, formation
of pairs, territorial defense) rather than to
the process of spermiogenesis. The local
mechanisms in seminiferous tubules were
probably sufficient for maintaining the intra-
tubular androgen concentrations essential
for normal spermatogenesis in mammals
(see Amann, 1983 for a review) and prob-
ably also in birds (Sharp et al, 1977) in
spite of low T levels in peripheral circula-

Plasma T₃ and T₄ levels in ganders
(2.7–9.7 nmol/l and 22.1–36.4 nmol/l resp)
were similar to those reported for other
species of Anseriformes (Jallageas et al,
1978; John and George, 1978; Campbell
and Leatherland, 1980) and for immature
domestic geese (Lazar et al, 1981). The
role of the thyroid gland in the develop-
ment of long day refractoriness (Dawson,
1984; Nicholls et al, 1984) and in the periodic replacement of plumage in birds (see Payne, 1972, for review) has been suggested. An involvement of sex and thyroid hormones in the control of moult was established largely in studies using pharmacological doses of exogenous hormone administration (Payne, 1972). Moreover, significant correlations between spontaneous changes in plasma sex and thyroid hormones and the feather moult were found in Barheaded geese (Dittami and Hall, 1983) and in laying hens (Herremans et al, 1988; 1989). In our experiment, the increase in plasma T4 levels was recorded from mid-February to mid-March, ie during the period of a marked drop of plasma T levels, and remained high throughout the breeding season. In mallards, plasma T4 also begins to increase after plasma T levels fall (Haase and Paulke, 1980; Sharp et al, 1986a). Increased levels of plasma T4 found during the breeding season are in accordance with findings in Canadian geese (John and Georg, 1978) and indicate an involvement of the thyroid gland in the control of metabolic processes and overall activity connected with reproduction. The peak in plasma T3 may be associated rather with the low temperature prevailing during this period.

Because no clear relationship between plasma levels of T, T3, T4 and remex moult was evident on a temporal basis, this relationship was analyzed by relating hormone concentrations to individual stages of remex moult. By this approach low plasma T levels were revealed at all stages of primary moult and regrowth. This finding corresponds with the classical antimoult role of testosterone (Payne, 1972) and suggests that the decrease of circulating androgen levels is a prerequisite to the onset of feather moult. This result physiologically parallels the drops in progesterone, necessary to induce mouling in laying hens as documented by Herremans et al (1988, 1989). The lowest T4 levels were found in ganders during the regrowth of primaries (stages 3–7), when plasma T3 levels were increased. On the other hand, high plasma T4 levels and low T3 concentrations were found before the moult and again during the final stages of primary replacement (stages 8–10). This inverse relationship clearly suggests that the rate of peripheral conversion of T4 to T3 may be important for feather growth.

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