Comparative changes in plasma concentrations of progesterone, oestradiol and LH during the ovulatory cycle in a multiple ovulating male line and a single ovulating traditional line of turkeys

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The aim of this study was to compare the profile of circulating concentrations of LH, progesterone and oestradiol in a multiple ovulating male line with that of a single ovulating line of traditional turkeys. Plasma samples from seven traditional and 12 male-line turkeys were obtained every 3 h for 36 h. Male-line and traditional turkeys had single peaks of LH and progesterone that were of similar duration in both lines. The mean height of the plasma peaks of LH and progesterone were similar in the two lines and there was no detectable peak plasma oestrogen concentration. Mean plasma concentrations of LH and oestrogen were higher in single compared with multiple ovulating turkeys, whereas there were no differences in mean plasma progesterone concentrations. The results indicate that the multiple ovulation state in genetically selected high-growth lines of turkey may be the result of a correlated response in the steroidogenic capacity of ovarian tissue associated with low plasma concentrations of oestrogen rather than of a disturbance in the hormone profile of the ovulatory cycle.

Introduction

The ovarian follicular hierarchy of avian species consists normally of a single series of follicles of increasing size that will be ovulated on separate days after achieving maturity. For a turkey laying an egg each day, the ovary should contain no more than nine or ten of these hierarchical follicles (Hocking, 1987), although some turkeys will have fewer follicles and will not lay on consecutive days. In contrast to traditional turkeys, modern lines that have been genetically selected for high growth rates have relatively large ovaries with many more maturing ovarian hierarchical follicles (Hocking, 1992; Melnychuk et al., 1997). The presence of two or more follicles at each position in the hierarchy results in the ovulation of two or more follicles in a single day. Many of the eggs that result from multiple ovulations are not suitable for incubation because of the increased proportion of double-yolked, soft-shelled and misshapen eggs that are laid (Hocking and Bernard, 1998). A significant proportion of ova from multiple ovulations is not collected by the infundibulum and is lost into the body cavity, where the triglyceride they contain is absorbed (Hocking, 1996). It is not known whether multiple ovulations occur separately throughout the day or at the same time.

A preovulatory peak in LH occurs 4–8 h before ovulation in turkeys and is accompanied by an increase in the concentration of progesterone in the plasma (Hammond et al., 1981; Sharp et al., 1981; Yang et al., 1997). Opel and Arcos (1978) measured plasma oestradiol concentrations throughout the ovulatory cycle in turkeys, but found no consistent pattern of changes in relation to ovulation. The effect of a multiple follicular hierarchy on the plasma steroid hormone profile during the ovulatory cycle has not previously been investigated in turkeys. Sharp et al. (1976) reported that there was no increase in the number of peaks in LH concentration during the ovulatory cycle of a strain of White Leghorn chickens selected for a high incidence of multiple ovulations compared with a commercial laying strain. Sharp et al. (1976) concluded that the multiple ovulations were attributable to a defect in the development of the follicular hierarchy rather than to a disturbance of the profile of circulating LH concentrations.

A comparison of the hormonal profile throughout the ovulatory cycle of turkeys that produce single or multiple ovulations has not yet been conducted. Therefore, a serial sampling experiment was performed to compare plasma oestradiol, progesterone and LH concentrations throughout the ovulatory cycle in a single ovulating traditional line and a multiple ovulating male line of turkeys. The aim of the study was to detect any differences in the plasma hormone profiles between the two strains during the ovulatory cycle, and to test the hypothesis that multiple ovulations are associated with multiple pre-ovulatory LH peaks throughout the ovulatory cycle or with a prolonged increase in LH. Both of these situations would indicate that ovulations occur at different times throughout the day. Alternatively, if

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the pre-ovulatory LH peak in the male line resembled that of
the single ovulating traditional line, it would imply that
several follicles were ovulated together in response to a
single LH peak. In spite of the excessively large quantity of
ovarian tissue and hierarchical follicles in the male line,
plasma oestrogen concentrations are higher in traditional-
compared with male-line hens (Buchanan et al., 1999,
2000). A second objective of the present study was to
determine plasma oestrogen concentrations to assess the
hypothesis that high concentrations at some point during
the ovulatory cycle are responsible for the higher pre-
valence of vaginal prolapse in the male line, as discussed by
Buchanan et al. (1999). Ideally, plasma concentrations of
FSH would also have been determined but the only
available avian assay (Krishnan et al., 1993) does not
measure turkey FSH.

Materials and Methods

Animals

The strains of turkeys (Meleagris gallopavo) used in these
experiments were the Big 6 male line, obtained from British
United Turkeys Ltd (Chester) and the Nebraska Spot, a
traditional line maintained at the Roslin Institute. Thirty
female turkeys from each line were obtained at 1 day old
and reared in floor pens containing wood shavings. They
had constant access to water and were fed a conventional
turkey diet provided in suspended tubular feeders. The
photoperiod was 14 h light:10 h dark (light from 02:00 to
16:00 h) for the first 18 weeks, 7 h light:17 h dark for the
next 11 weeks, followed by photostimulation at 29 weeks
and 4 days with 14 h light:10 h dark to stimulate sexual
maturity. At 18 weeks of age, four turkeys from each strain
were housed in six pens, in a randomized block design.
Individual egg production was recorded by feeding the
turkeys capsules containing fat-soluble dyes three times per
week. Gelatin capsules (size 4, Agar Scientific Ltd, Stansted,
Essex) were half filled with Sudan black or Sudan III (red)
and fed by gavage three times per week. The traditional
turkeys received one capsule and the male line received
two capsules because of the dilution effect of their greater
body weight. Within a pen, one turkey was given black, one
turkey was given red, one turkey was given alternating red
and black and one bird was handled but not fed a capsule.
Eggs were collected daily, boiled in a waterbath for 20 min
and then cut in half. The colour of the dyed rings in the
yolks of the eggs enabled the egg production of each hen to
be determined. Within each pen, the turkeys with the most
regular egg production were selected for the experiment,
as they were most likely to ovulate during the sampling period.
As the laying pattern of the male line was more irregular
than that of the traditional line, 12 male-line and seven
traditional-line turkeys were used for the experiment.

The sampling period for plasma collection started 6
weeks after the onset of photostimulation. The turkeys were
handled daily in the week before the sampling period to
allow them to become accustomed to handling, so that the
interference caused by the sampling was less likely to delay
oviposition. Whole blood was obtained from each turkey
by superficial venepuncture of the brachial vein and trans-
ferred to blood collection tubes coated with 5 ml heparin.
The samples were centrifuged at 2000 g for 10 min and the
plasma was removed and stored in 1.5 ml Eppendorf tubes
at –20°C. Each of the selected turkeys was sampled every
3 h for 36 h starting at 10:00 h. The sampling took about 1 h
and the turkeys were always sampled in the same order
to ensure a period of 3 h between each sample. During
the hours when the lights were off, spectacle type head
torchers (RS Components Ltd, Corby) were used for the
sampling to minimize disruption of the lighting schedule.
Egg production was recorded hourly for the final 24 h of the
sampling.

Turkeys were killed 12 h after the final blood sample
was collected by an overdose of sodium pentobarbitone
(Veterinary Drug Company, Falkirk). The abdominal cavity
was opened and the ovary removed from the body cavity.
Yellow ovarian follicles were cut from the residual ovary
and follicles > 0.5 g were classed as hierarchical ovarian
follicles, counted and weighed. The postovulatory follicles
were removed and counted, and the residual ovary was
weighed. The sequence of previous ovulations was esti-
mated by arranging the postovulatory follicles according
to size, with follicles of similar size grouped together by
the same person as likely multiple ovulations. These data
supplemented the egg production data and allowed com-
parison of the ovulation pattern to the hormone profile
for each bird. The mass of the residual ovary was also
recorded.

Radioimmunoassays

The plasma concentrations of oestradiol, progesterone
and LH were measured by classical double antibody radio-
immunoassays. Each assay was validated for use on turkey
plasma by serial dilution of single samples to check for
parallelism with the standard curve. Samples for each
hormone were analysed in single assays to eliminate inter-
assay variability. Oestradiol concentrations were deter-
mined in duplicate using the method of Webb et al. (1985).
The plasma samples were extracted initially using an
antibody–Sepharose extraction procedure because of the
high fat content of laying turkey plasma. The efficiency of
the extraction procedure was 45.7%. The mean intra-assay
coefficient of variation was 15.2% and the lowest sensitivity
of the assay was 1.5 pg per tube. The concentration of
progesterone was measured in duplicate as described by
Law et al. (1992). The samples were analysed in a single
assay with an intra-assay coefficient of variation of 12.1%
and lowest sensitivity of 25 pg per tube. The plasma
concentration of LH was determined in triplicate as
described by Sharp et al. (1987). The intra-assay coefficient
of variation was 12.3% and the lowest sensitivity of the
assay was 0.045 ng per tube.
Statistical analyses

Two-sample unpaired Student’s t tests were used to compare the mean hormone concentrations over the sampling period and the ovarian characteristics for each strain. The height of the peak in hormone concentration was calculated as the difference between the highest concentration and an average baseline value for each bird. The duration of each peak was estimated as the difference in sample time from the start of the steep increase in concentration to the time when the concentration had returned to baseline values. The mean height and duration of the peaks in progesterone and LH were compared using two-sample unpaired Student’s t tests. A one-sample t test was used to test whether the time difference between the peak in progesterone and the peak in LH was significantly different from zero, and a two-sample t test was used to compare the time difference between the two lines of turkeys.

Results

Eggs were obtained from five of the seven traditional-line and four of the 12 male-line turkeys during the sampling period. Postmortem examination of the records of eggs laid after the sampling period and the presence of ovulated follicles in the oviduct when the birds were killed indicated that all of the traditional-line and nine of the 12 male-line turkeys had ovulated during the sampling period.

The male line had significantly more postovulatory follicles than did the traditional line (Table 1). Male-line turkeys also had a significantly greater incidence of two or more postovulatory follicles at the largest position (Table 1).

Typical traces of the changes in plasma oestradiol, progesterone and LH throughout a 36 h period in a traditional-line and a male-line turkey that had ovulated during the sampling period are shown (Fig. 1a,b). Plasma LH peaks were clearly visible in ten of the male-line and all of the traditional-line turkeys. One of the male-line turkeys showed a distinct LH peak, although no ovulated follicles were present in the oviduct (Fig. 1c) and two male-line turkeys showed no evidence of a peak for any of the plasma hormones measured (Fig. 1d).

No turkey from either strain showed more than one peak in the plasma concentration of LH during the sampling period. The mean height and duration of the peak and the

<table>
<thead>
<tr>
<th>Postovulatory follicles</th>
<th>Traditional line</th>
<th>Male line</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3.1 ± 0.34</td>
<td>7.9 ± 1.15</td>
<td>*P &lt; 0.01</td>
</tr>
<tr>
<td>Largest (similar size)</td>
<td>1.0 ± 0.00</td>
<td>2.0 ± 0.19</td>
<td>*P &lt; 0.001</td>
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Table 1. Mean (± SEM) number of postovulatory follicles and number of postovulatory follicles of the largest size in traditional line (n = 7) and male line turkeys (n = 12)

Fig. 1. Changes in plasma concentration of oestradiol (△), progesterone (●) and LH (○) at 3 h intervals during a 36 h sampling period in turkeys. (a) Traditional-line turkey that ovulated during the sampling period. (b) Male-line turkey with a recorded ovulation. (c) Male-line turkey in which no oviposition was recorded and an internal ovulation is assumed to have occurred. (d) Male-line turkey in which there was no evidence for oviposition or ovulation. Bar at the top of the figure represents the photoperiod (open) and scotoperiod (filled).
overall mean plasma concentration of LH for the two strains are shown (Fig. 2a). The mean plasma LH concentration was significantly greater in the traditional line than in the male line (P < 0.001), whereas there were no differences in the mean height or duration of the LH peaks between the two strains.

Plasma progesterone concentration showed a preovulatory increase that coincided with the increase in plasma LH in both the traditional- and male-line turkeys (Fig. 1a,b). No such increase was visible in either of the male-line turkeys that did not ovulate during the sampling period (Fig. 1d). Progesterone concentrations reached a peak significantly later than LH concentrations (2.0 ± 0.5 h, P < 0.001) but there was no difference in this time period between the two strains. There was no significant difference in the duration of the progesterone peak between the two strains (Fig. 2b). The progesterone peak lasted slightly longer than the LH peak in both strains, but this difference was not statistically significant. The mean plasma progesterone concentration and mean height of the progesterone peak in the traditional- and male-line turkeys are also presented (Fig. 2b): neither the mean plasma concentration nor the mean plasma peak of progesterone were significantly different between the traditional and male lines.

There was no consistent pattern of changes in plasma oestradiol concentration in either the traditional- or the male-line turkeys. Some individuals showed peaks in plasma oestradiol around the same time as the LH peak (Fig. 1a,b), in others peaks occurred before or after the LH peak and in some there was no distinct oestradiol peak. The mean plasma oestradiol concentration was significantly lower in the male line compared with the traditional line (P < 0.001), as was the mean of the highest concentrations (P < 0.05) (Fig. 3b).

The male line had significantly more hierarchical follicles (P < 0.001) and a significantly greater residual ovary mass (P < 0.001) compared with the traditional line (Fig. 3a).

Discussion
The data from the 36 h sampling period showed peaks in plasma progesterone and LH associated with ovulation in both traditional- and male-line turkeys. These findings are in agreement with previous findings in turkeys (Hammond et al., 1981; Sharp et al., 1981) and domestic hens (Etches, 1996a). The lack of consistent patterns of oestradiol concentrations during the ovulatory cycle in either strain of turkey is also in agreement with previous results (Opel and Arcos, 1978).

The LH peaks were no more frequent in the male line.
than they were in the traditional line, indicating that multiple follicles are ovulated in response to single pre-
ovulatory peaks of LH and progesterone. This finding is
similar to those reported by Sharp et al. (1976) that multiple
ovulations occurred in the domestic hens after single LH
peaks.

Multiple ovulations occurred in the male line during the sampling period as indicated by the presence of two or
more fresh postovulatory follicles, although these were not
necessarily associated with yolks in the oviduct or in laid
eggs. The abdominal cavity of male-line turkeys frequently
contained intact ovulated follicles or yolk fluid that was
assumed to have come from ova that were being absorbed.
The results indicate that a significant proportion of ovulated
follicles is lost as internal ovulations into the abdominal
cavity in male-line turkeys. No more than one egg was
collected from each turkey during the 36 h period, confirm-
ing that the additional postovulatory follicles in the male
line must represent follicles lost through internal ovulation
or eggs with defective shells that were destroyed before they
could be collected.

Most of the eggs laid during the sampling period
contained yolks that were ovulated before the start of the
sampling period and it was not possible to calculate the
time between ovulation and subsequent oviposition. In
general, the results did not show a clear relationship
between oviposition and ovulation, such as occurs in fowl
(Etches, 1996a), in which ovulation tends to occur
30–45 min after oviposition of the previous egg. A longer
period of recording egg production after the sampling
period would be necessary to determine the time interval
between ovulation and oviposition. In the present study, it
was necessary to record the presence of fresh postovulatory
follicles to establish whether ovulation had occurred and a
longer egg collection period was not possible.

The presence of gaps in the hierarchy corresponding to
days when no follicle was recruited is more common in
male-line than it is in traditional-line turkeys (Hocking
and Bernard, 1998). The fact that such a gap was not observed
in traditional-line turkeys in the present study may be
coincidental or a consequence of prior selection for regular
oviposition. The absence of peaks for LH and progesterone
in ovulatory cycles without an observed or inferred
ovulation is consistent with the conclusion that follicular
growth and recruitment occur normally in multiple
ovulating birds but that more than one follicle is likely to be
recruited in any one period.

The peaks in plasma progesterone and LH were no more
prolonged in the male line than they were in the traditional
line, which further supports the hypothesis that several
follicles are released in response to a single LH surge in the
multiple ovulating strain. It seems likely that these follicles
are released around the same time, relative to the time of
peak plasma LH concentration. If the follicles were ovulated
many hours apart, several peaks or an extended increase in
LH might have been observed in a multiple ovulating line.
The release of several follicles from the ovary at a similar
time during the ovulatory cycle may make it difficult for the
infundibulum to capture all of the follicles that are ovulated.
If the oviduct did capture more than one follicle, this is
likely to lead to the formation of double-yolked, misshapen
or soft-shelled eggs (van Middelkoop, 1971; Hocking et al.,
1987).

There was no clear pattern of changes in plasma
oestradiol concentration throughout the ovulatory cycle in
turkeys. The results did not provide any evidence for higher
peak oestradiol concentrations in the male line than in the
traditional line. When the mean plasma oestradiol concen-
tration over the 36 h sampling period was compared between
the two strains, plasma oestradiol was lower in the male
line, confirming earlier reports of single-point estimates
(Buchanan et al., 1999, 2000).

The present results showed that plasma LH was lower in
the multiple ovulating male line of turkeys, in contrast to the
absence of differences in the mean plasma concentrations
of LH between single and multiple ovulating groups of
domestic hens (Sharp et al., 1976). Williams et al. (1986)
reported a significant difference in plasma LH concentration
between fat-line and lean-line chickens, with a greater LH
concentration in the lean line. It is unclear why selection for
different body traits, such as increased abdominal fat or
increased growth rates, should alter plasma LH concentra-
tion. It is possible that the capacities of the hypothalamus or
pituitary of male-line turkeys have not increased to produce
circulating plasma hormones in proportion to the increased
body size of the strain. Alternatively, differential sensitivity
of the hypothalamus and pituitary to stimulatory or
inhibitory signals may be involved. Food restriction changes
the sensitivity of the pituitary to cLH-releasing hormone I,
and possibly gonadal hormones, during the laying period
(Bruggeman et al., 1998), and genetic changes associated
with selection for growth rate and body composition might
have had a similar effect.

No differences were found in the mean progesterone
concentrations over the 36 h sampling period between the
two strains, consistent with results obtained at a single
time point (Buchanan et al., 1999, 2000). In both strains,
progesterone increased to two to four times its basal
concentration in a preovulatory peak that occurred slightly
later than the preovulatory LH peak. This finding is
consistent with that of Sharp et al. (1981) who showed that
there was a progesterone peak about 4 h after LH in the
domestic fowl. A more frequent sampling regimen would be
required to determine whether the duration of increased
progesterone concentration is significantly longer than the
duration of increased plasma LH concentration.

From studies in domestic hens, it is thought that the onset
of the preovulatory surge of progesterone stimulates the
release of LH from the hypothalamus in a positive feedback
mechanism that eventually results in ovulation, which is
followed by a decrease in the release of LH as a result of
negative feedback from high plasma steroid concentrations
(Etches, 1996b). The ovary is the major source of pro-
gesterone and oestradiol: the largest follicles produce
progesterone whereas the smaller follicles and residual ovary produce predominately oestrogens (Armstrong, 1984; Porter et al., 1991; Buchanan et al., 2001). Therefore, it was surprising that the male-line turkeys, which have many more mature follicles and a much bigger residual ovary, do not have higher plasma concentrations of progesterone and oestradiol compared with the traditional line. The results indicate that the steroidogenic capacity of the ovarian cells in the large ovary of the male line may be poor and are consistent with studies of individual ovarian follicles in vivo (Buchanan et al., 2001). Poor synthesis of oestrogen by the ovarian cells resulting in a decrease in plasma hormone concentration may affect the sensitivity of the hypothalamus and anterior pituitary, resulting in increased FSH secretion, overstimulation of ovarian growth and increased recruitment into the follicular hierarchy. The presence of lower plasma LH concentrations in the male line is consistent with this hypothesis. Alternatively, it is possible that several follicles of the largest size are required to produce sufficient progesterone to trigger ovulation in the male line. If this were the case, then genetic selection may have inadvertently resulted in the development of the typical multiple follicular hierarchy via selective breeding from females with two or more mature follicles that collectively produced enough progesterone to cause ovulation in birds with decreased sensitivity of the hypothalamus to gonadal steroids. Genetic selection for high growth rates and meat yields in turkeys may have led to an increase in the propensity for multiple ovulation by at least two routes: firstly, by overstimulation of ovarian growth as a result of a lack of control by negative feedback of oestrogens (the peripheral, or decreased gonadal steroid hypothesis) and, secondly, by requiring the progesterone output of several follicles for ovulation to occur (the central, or decreased hypothalamic–pituitary sensitivity hypothesis). However, genetic selection may act primarily through changes in local growth factor expression in the ovary, hypothalamus and pituitary that result in altered sensitivity of these tissues to regulatory signals, as has been well documented for the insulin-like growth factor (IGF) system (Lackey et al., 1999).

In summary, male-line turkeys that are characterized by multiple ovulations do not ovulate in response to multiple or extended peaks in the plasma concentrations of LH or progesterone. There was no difference in the height of the plasma peaks in LH between male-line and traditional-line turkeys, whereas the mean plasma concentration of LH was higher in traditional- than it was in male-line turkeys. The mean plasma peaks and the overall mean plasma concentrations of progesterone for the two lines were not significantly different. There was no detectable peak in plasma oestrogen concentration during the ovulatory cycle, and the mean plasma concentration of oestrogen was lower in male-line than it was in traditional-line turkeys. The multiple ovulation state in genetically selected high-growth lines of turkeys may be caused by a correlated response in the steroidogenic capacity of ovarian tissue associated with low plasma concentrations of oestrogen and a change in the sensitivity of the hypothalamus–pituitary axis.

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