ABSTRACT  Testosterone profiles and semen characteristics were determined using yearling and adult captive wild-strain Mallard (Anas platyrhynchos) drakes. Wild-strain Mallard hens were artificially inseminated by modifying a technique developed for domesticated poultry. In both adult and yearling drakes, there was a change in the concentration of circulating plasma testosterone during the reproductive season. Testosterone concentrations increased from basal levels in March, peaked in April, and decreased to basal levels in May. The decrease in testosterone concentration to basal level was 2 wk earlier in yearlings than in adults ($P < 0.05$). The decrease in testosterone concentration was associated with the onset of postnuptial molt. Semen volume (0.04 to 0.08 mL) and semen concentration ($\sim 1.32 \times 10^9$ spermatozoa per milliliter) were not different between adult and yearling drakes ($P > 0.05$). Overall mean fertility for yearling and adult drakes obtained with artificial insemination was 70.4%. These results suggest that artificial insemination may be used successfully in the propagation of captive wild-strain Mallard ducks.

(Key words: semen, testosterone, artificial insemination, Mallard)

INTRODUCTION

The Mallard duck (Anas platyrhynchos) is a popular game bird that is widely distributed in North America. Predation and loss of breeding habitat have contributed to a population decline (Johnson and Shaffer, 1987; Reynolds et al., 1990; Greenwood et al., 1995). Large-scale releases of game-farm Mallards have been proposed as a means to reestablish the stocks (Cheng et al., 1980). However, there is concern that Mallards of game farm origin have morphological and physiological traits that are genetically different from the wild population as a result of having been selected for high egg production over many generations. In contrast, captive propagation programs of wild-strain Mallards are often hindered by the failure of birds to achieve their full reproductive potential (Phillips and van Tienhoven, 1960; Phillips, 1964; Cheng et al., 1980; Bluhm et al., 1983).

Mallard drakes breed as yearlings; however, they show reduced productivity compared with their older counterparts. Reproductive inferiority of young birds has been attributed to lack of reproductive experience, lack of physical development, or both (Bruggers and Jackson, 1981; Blohm, 1982; Curio, 1982). As circulating androgen levels are a reflection of the timing and extent of gonadal development, a comparison of testosterone profiles and semen characteristics between yearling and adult drakes may determine whether the poor reproductive success of yearling drakes is due to copulatory inexperience or lack of testicular development.

Although the annual reproductive cycles in male and female Mallards have been well characterized (Paulke and Haase, 1978; Donham, 1979; Haase, 1983), virtually no studies have compared the relationship between age and blood testosterone levels in drakes. In Mallards exposed to natural lighting, the breeding season is restricted to the spring. During the winter, birds become photosensitive and in this state respond to increasing photoperiod with gonadal growth and increasing plasma testosterone levels (Haase, 1983). Circulating testosterone levels peak and the testes are fully developed during the breeding season in April and May (Paulke and Haase, 1978; Haase et al., 1985). In late spring and early summer, birds become photorefractory, as reproductive activity is not maintained by long days. This response corresponds to a rapid decline in circulating concentrations of testosterone and regression
of the testes (Haase et al., 1985). The decline in plasma concentrations of testosterone is correlated with postnuptial molt at the end of which drakes are rendered flightless for 2 to 3 wk due to loss of their primary flight feathers (Johnson, 1961). After postnuptial molt, the drake can no longer be distinguished from the hen on the basis of plumage color, as the drake’s characteristic green head feathers are replaced by brown feathers. When the growth of new flight feathers is completed, the prenuptial molt begins (Johnson, 1961). The testes appear to be completely regressed by the time that complete prenuptial plumage is acquired (Johnson, 1961) and the drakes have returned to their characteristic breeding plumage. A second peak in testosterone occurs in late fall, but unlike the first is not accompanied by an increase in testes size; rather, it is thought to be related to pair bond formation (Paulke and Haase, 1978).

Programs to help reestablish the wild population through captive propagation and release would benefit from an improved understanding of the basic breeding biology and an ability to enhance the breeding process by the application of special techniques of artificial breeding such as artificial insemination (AI). With the advent of AI it would be possible to introduce genetic variability into small captive or wild populations by transporting semen from distant populations. Accordingly, the risk of introducing parasites and diseases associated with foreign birds and the problems connected with the transport of live birds is reduced. Specific objectives of this study were to compare plasma testosterone profiles and semen characteristics between yearling and adult Mallard drakes and to determine whether AI could be used as an alternative management technique in the rearing of captive wild Mallards.

MATERIALS AND METHODS

Breeding Stock

Genetically wild Mallards used in this study were obtained from the Delta Waterfowl Research Station, Portage la Prairie, MB, Canada. Mallards had been hatched, reared, and maintained using standard procedures outlined by Ward and Batt (1973) from eggs salvaged from agricultural fields in south central Manitoba.

Care and Management of Birds

On February 14, 1995, drakes (10 yearlings and 13 adults) were assigned to outdoor cluster pens (siblings and brood mates were separated) at the University of British Columbia’s San Rafael Research Aviary in BC Canada. Drakes were separated by age and kept approximately six to a pen. Each pen (approximately 2.4 m × 3 m) contained areas of open water and dry land (gravel). A 1.2-m plywood wall around the base of the pen shielded the birds from outside disturbances. On March 28, hens (n = 16) were individually assigned to pens (approximately 2.4 m × 1.5 m) adjacent to drakes. Birds in separate pens had visual and auditory exposure to each other, but were physically separated by chain-link fencing. All birds had ad libitum access to a commercially available duck pellets, supplemented with wheat and oyster shell. Hens were given access to straw-filled wooden nest boxes.

Semen Collection and Evaluation

Semen collections were done between 1300 and 1500 h Pacific Standard Time, within drake pens. Each drake was caught by hand. The method of semen collection followed the massage procedure outlined by Lake and Stewart (1978) with the following modifications. Semen was collected by a single seated technician, with the drake held with its head toward the technician and his wings held between the technician’s knees. The tube used for catching the semen was held between the fingers of the hand used to massage the abdomen. The opposite hand gently stroked the back toward the tail ending with the thumb and forefinger on each side of the cloaca. Gentle pressure was sometimes applied around the cloacal region to extrude the phallus (Cheng and Otis, 1982).

Drakes used as semen donors (n = 23) were trained twice a week for about 3 wk before inseminations were begun. This training involved handling and attempting to ejaculate the birds. After that, drakes were routinely collected from twice a week for 12 wk. Care was taken to minimize contamination of semen with uric acid waste. The variables to assess and compare semen characteristics of individual yearling and adult Mallard drake semen samples included: concentration using a hemocytometer, motility score, and appearance score as described by Lake and Stewart (1978). Briefly, motility was ranked on a scale of 1 to 5, where 1 represented semen with < 20% motility and 5 represented semen with > 80% motility. Appearance was also subjectively scored on a scale of 1 to 5, with the bottom of the scale representing watery semen and the top of the scale representing thick white semen. The amount of semen produced was small; therefore, volume was assessed by weighing samples and assuming the density of semen to be 1 mg/mL (Etches, 1996). Semen used for inseminations was pooled by age group.

Insemination

A 1-mL tuberculin syringe was used for inseminations directly into the oviduct of laying birds. About 0.1 mL of air was first drawn into the syringe to be able to push out the whole volume of semen (0.05 mL; ∼ 6.8 × 10⁷ spermatozoa) at the time of insemination. To prevent injury, insemination was not attempted until a hen had laid at least one egg. It was difficult to evert the oviduct, as is commonly done with domestic chickens and turkeys; therefore, the syringe was gently inserted on the left side of the cloaca and somewhat dorsally until the oviduct was found. The syringe was then gently inserted one or two
centimeters and the semen deposited. Yearling (n = 8) and adult (n = 8) hens were inseminated within 1 h of collection with pooled semen from either adult or yearling drakes.

**Egg Collection and Incubation**

Nest boxes were examined once a day. Eggs were removed from the nest boxes as they were laid and replaced with dummy chicken eggs to maintain the normal egg laying pattern. Dummy clutches were removed 5 d after the last egg was laid. Collected eggs were stored for 1 wk in a cool cupboard during which they were rotated daily. Eggs were washed with an antibacterial solution just before incubation in a portable forced draft incubator at 37.6 C and 21.1 C wet bulb temperature. Eggs were automatically rotated hourly through 90°.

Eggs were candled at 7 d to determine whether development was proceeding. Questionable eggs were removed from the incubator and opened to ascertain whether they were fertile. Two days before the expected hatch date, eggs were placed into a hatching tray at the bottom of the incubator. Dead embryos detected during additional candlings were recorded, aged by the technique of Caldwell and Snart (1974), and classified as early-dead (≤ 7 d), mid-dead (8 to 18 d), or late-dead (≥19 d). Mortalities were also classified into one of seven malposition groups (Fant, 1957).

**Blood Collection**

Blood was collected every 2 wk between February and May, 1995 from the 23 drakes (10 yearlings, and 13 adults) used for AI. Blood samples (1 to 1.5 mL) were collected between 1200 and 1600 h from the medial wing vein using sterile 3 cc syringes and 22-gauge needles. Samples were transferred to individual 3-mL heparinized vacutainer tubes2 and centrifuged at 1,250 g for 5 min within 30 min of collection; the plasma was aspirated and stored at ±20 C until assayed for testosterone.

**Radioimmunoassay**

A commercial RIA kit3 was used for testosterone assay. Samples were run in duplicate. The kit used a solid-phase RIA based on polypropylene tubes coated with antibodies to testosterone. 125I-labeled testosterone competed for a fixed period of time with testosterone in the plasma sample for antibody sites. Once the supernatant was decanted (to separate bound from free testosterone), the tube was counted in a gamma counter to yield counts per minute. These counts were converted to the concentration of testosterone in the sample using a calibration curve. The RIA kit is equipped with human serum-based calibrators ready to use, having testosterone values ranging from 0 to 16 ng/mL. The assay can detect as little as 0.04 ng/mL.

### Statistical Analyses

Trait means between yearling and adult drakes were compared using t tests. Plasma testosterone values were analyzed as univariate repeated measures analysis for a split-plot design. Differences in treatment means were separated by least squares analysis of variance procedure using General Linear Models procedure of SAS® Institute (Littell et al., 1991). The model used was:

\[ Y_{ijk} = \mu + A_i + D_{ij} + T_k + (AT)_{ik} + e_{ijk} \]

where \( i = 1...2; j = 1...8; k = 1...23; \mu = \) the general mean; \( A_i = \) the effect of the \( i \)th age; \( D_{ij} = \) the whole-plot error for drakes within age; \( T_k = \) the effect of the \( k \)th sampling time; \( (AT)_{ik} = \) the interaction between age and sampling time; and \( e_{ijk} = \) the split-plot error on repeated measures. The whole-plot error was used to test age effects. The other effects (sampling time and interaction of age with sampling time) were tested using the split-plot error.

Chi-square analysis with Yates correction for continuity (Walpole, 1982) on 2 x 2 contingency tables was used to test for drake effects on egg fertility. Fertility was expressed as the percentage of viable embryos on Day 7 of incubation. Hatchability was defined as the percentage of eggs hatched per fertile eggs incubated. Due to the limited number of observations, hatchability and embryo mortalities were not statistically analyzed. Unless otherwise stated, all comparisons were made at \( P < 0.05 \).

### RESULTS

Semen could be collected from 18 (9 yearlings and 9 adults) out of 23 drakes. The ability to obtain semen from individual birds was highly variable. At some collection periods semen could not be obtained from all drakes. If semen did not appear after 60 s of massage of any drake, it was unlikely that further massage would have been effective.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yearling</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, mL</td>
<td>0.04 ± 0.01 (23)</td>
<td>0.08 ± 0.02 (26)</td>
</tr>
<tr>
<td>Appearance score</td>
<td>3.1 ± 0.2 (22)</td>
<td>3.3 ± 0.2 (20)</td>
</tr>
<tr>
<td>Motility score</td>
<td>2.8 ± 0.3 (21)</td>
<td>3.5 ± 0.3 (19)</td>
</tr>
<tr>
<td>Concentration, × 10⁹/mL</td>
<td>1.32 ± 0.29 (18)</td>
<td>1.32 ± 0.31 (21)</td>
</tr>
</tbody>
</table>

1Values are overall means ± SEM of individual samples from nine yearling and nine adult drakes subjected to nine collections periods. \( n = \) total observations.

2Volume, appearance, motility, or concentration between the two age groups for each collection period or over the course of the study did not differ significantly \( (P > 0.05) \).

3Appearance and motility of semen were subjectively scored on scales of 1 to 5, with the high end of the scales representing good quality semen.
ARTIFICIAL INSEMINATION IN CAPTIVE MALLARD DUCKS

Profiles of plasma testosterone concentrations are presented in Figure 1. Testosterone mean concentrations ranged from 0.02 to 1.59 ng/mL during the 4-mo collection period. Testosterone concentrations were not significantly different between yearling and adult drakes ($P > 0.05$), but there was considerable variation in testosterone concentrations between individual birds. Testosterone concentrations were significantly influenced by time ($P < 0.01$), increasing from a low of 0.13 ng/mL in February to a high of 1.26 ng/mL in early April. In May, testosterone concentrations declined. On April 25, yearling drakes had significantly lower plasma testosterone concentrations than adult drakes ($P < 0.01$). The respective means for plasma testosterone concentrations were $0.55 \pm 0.28$ and $1.59 \pm 0.25$ ng/mL for yearling and adult drakes. By May 9, plasma testosterone concentrations for yearling ($0.61 \pm 0.29$ ng/mL) and adult ($0.99 \pm 0.27$ ng/mL) drakes were not different ($P > 0.05$).

The fertility and hatchability data is presented in Tables 2 and 3. The fertility of individual hens ranged from 25 to 100%. Hatchability ranged from 0 to 67%.

### FIGURE 1. Plasma testosterone concentrations for yearling and adult Mallard drakes during the reproductive season. Values are presented as least squares means ± SEM. Values within time points with no common letters differ significantly ($P < 0.05$).

![Testosterone concentrations graph](image)

<table>
<thead>
<tr>
<th>Hen</th>
<th>Hen age</th>
<th>Drake age</th>
<th>Clutch</th>
<th>Laying sequence after first and subsequent inseminations</th>
<th>Percentage fertile (%)</th>
<th>Percentage hatch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1</td>
<td>2</td>
<td>A</td>
<td>AI -O-FFF-F</td>
<td>80.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>AI NFOO-F-F</td>
<td>66.7</td>
<td>50.0</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>2</td>
<td>A</td>
<td>AI --FFFF-F</td>
<td>100.0</td>
<td>n/a</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>1</td>
<td>A</td>
<td>AI --O-00O-O</td>
<td>25.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>AI NFFPOO-O</td>
<td>57.0</td>
<td>50.0</td>
</tr>
<tr>
<td>J</td>
<td>2</td>
<td>2</td>
<td>A</td>
<td>AI --O-FF-F</td>
<td>100.0</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>AI O-FF-F-FO</td>
<td>60.0</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>AI O-FF--FO</td>
<td>60.0</td>
<td>0.0</td>
</tr>
<tr>
<td>K</td>
<td>2</td>
<td>2</td>
<td>A</td>
<td>AI OOFF-F-F</td>
<td>66.7</td>
<td>50.0</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>2</td>
<td>A</td>
<td>AI --FF--O</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>1</td>
<td>1</td>
<td>A</td>
<td>AI OF-F-FF</td>
<td>83.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72.6</td>
<td>53.3</td>
</tr>
</tbody>
</table>

1Hen and drake age is 1 = yearling and 2 = adult.
2O = egg not fertile egg; F = fertile egg; = no egg laid; N = eggs likely fertilized by previous insemination.
3n/a = fertile eggs not incubated to hatch.
TABLE 3. Effect of drake age (yearling vs adult) on fertility, hatchability and embryo mortality rates of Mallard eggs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yearling</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage fertile</td>
<td>58.8 (10/17)¹</td>
<td>75.7 (28/37)²</td>
</tr>
<tr>
<td>Percentage hatched</td>
<td>20.0 (2/10)</td>
<td>33.3 (6/18)²</td>
</tr>
<tr>
<td>Percentage mortality:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early-dead¹</td>
<td>50.0 (4/8)</td>
<td>33.3 (4/12)</td>
</tr>
<tr>
<td>Late-dead²</td>
<td>50.0 (4/8)</td>
<td>66.7 (8/12)</td>
</tr>
</tbody>
</table>

¹Values within rows with no common superscript differ significantly (P < 0.05).
²Ten out of 17 eggs fertile.
³Discrepancies between fertile and hatch egg numbers are due to fertile eggs not incubated until hatch.
⁴Early-dead represent mortalities < 7 d of incubation.
⁵Late-dead represent mortalities > 19 d of incubation.

Fertility of hens inseminated with pooled yearling or adult drake semen was 58.8 and 75.6%, respectively, with an overall mean of 70.4%. There was no significant (P > 0.05) difference between fertility rates for the two age groups.

**DISCUSSION**

This is the first known report of successful AI in hand-reared wild Mallards. The mean fertility obtained with AI over both age groups was comparable with that reported for naturally mated pairs (Batt and Prince, 1978). Fertility was previously found to be 50% for yearling pairs that were allowed to choose their own mates and mate naturally (Stunden et al., 1995). The production of live ducklings strongly suggests that AI can be used successfully to supplement natural mating in captive Mallards.

A feature of the hen’s reproductive tract is the ability to store semen in a viable state for extended periods of time after copulation or insemination. This storage ability allows for the fertilization of consecutively laid eggs between inseminations. Fertile eggs were obtained as much as 11 d after a single insemination (data not presented). This long period of fertility agrees with results reported by others (Elder and Weller, 1954; Lake, 1983). The results indicate that the period included from the 3rd to 7th d after an insemination is most likely to contain fertile eggs. With the spermatozoa concentration used in this study, hens needed to be inseminated at weekly intervals to maintain maximum fertility using AI.

One disadvantage of AI might be the failure to fertilize those eggs laid before insemination. The irregularity of the Mallard hens reproductive cycle makes it difficult to predict the date upon which an egg will be laid. Often hens will skip a day between egg laying, or stop laying completely for up to 3 wk between clutches. It may be possible to overcome this problem by inseminating before laying begins, as is done with several other avian species (Berry, 1972). Nest-building often occurs a few days before ovulation of the first egg and has been correlated to a readiness to copulate in many avian species (Lehrman, 1959). Therefore, nest-building could possibly be used as an indication of reproductive maturation.

Post-mortem examinations revealed that the bulk of mortalities occurred during the final stages of incubation and the majority of these dead embryos were in abnormal positions. Typically, the duckling was either rotated so that its beak was away from the air cell or the beak was over the right wing instead of under the wing. Deaths caused by malpositions that make hatching extremely difficult are usually attributed to unfavorable incubation conditions such as abnormal temperature or nutritional deficiencies in the hen’s diet (Etches, 1996). No such conditions were noted in this study, although incubator humidity readings did fluctuate.

The results of this study do not support a hypothesis that differences in semen characteristics contribute to the low reproductive success of yearling drakes. Semen collected from yearlings and adults did not differ with respect to the number of spermatozoa per milliliter, sperm motility, or semen volume. It is generally accepted that semen characteristics affect fertility (Allen and Champion, 1955).

Comparisons of ejaculates from wild Mallards with those reported by various authors for domesticated duck species (Kamar, 1962; Gvaryahu et al., 1984), suggest that quantitative characteristics are lower for semen obtained from wild Mallards. Kamar (1962) recorded a mean value of 3.63 × 10⁹ spermatozoa per milliliter in Sudani drakes and 5.85 × 10⁹ spermatozoa per milliliter in Pekin drakes. In contrast, Gvaryahu et al. (1984) reported a concentration of 1.35 × 10⁹ spermatozoa per milliliter for Muscovy drakes, which is similar to the concentration obtained in this study. It is possible that the lower concentration found in the present study may be due to the addition of ejaculatory-groove region fluid during collection. This region is considered to be an accessory reproductive organ in the drake (Fujihara et al., 1976). At copulation, fluid similar in composition to lymph is released and mixes with the semen ejected from the vas deferens. Such secretions have been found to have favorable effects on the fertility of fresh spermatozoa from the drake (Fujihara et al., 1976). As well, domesticated ducks have been selected for high reproductive performance and this may have altered the semen characteristics. Differences in degree of adaptation to captivity, breed, and method of collection between this experiment and that of the others cannot be ruled out as sources of variation.

Seasonal changes in the concentration of testosterone in the peripheral blood of Mallard drakes observed in this study agree with previous findings (Paulke and Haase, 1978; Donham, 1979). Mallard drakes exhibited a steep decrease in the concentration of testosterone in May, associated with the onset of the photorefractory period. Testosterone concentrations in drakes tended to be quite variable.
One of the most striking features of these results was the pattern of circulating testosterone concentrations decreasing to basal levels 2 wk earlier in yearling drakes than in adult drakes. It appears that adulthood delayed the onset of the photorefractory period. The results showed no trend for an overall increase in circulating testosterone concentrations in older birds, which is a pattern observed in many farm animals (Gunarajasingam et al., 1985).

Results presented provide evidence that AI may be used to supplement natural mating in Mallard pairs not willing or unable to mate naturally in captivity. Furthermore, the practice of semen collection and insemination should provide valuable insights into the reproductive physiology of wild Mallards. Finally, a logical extension of an AI program would be to preserve semen to allow the exchange of genetic material between distant populations and the establishment of gene banks.

ACKNOWLEDGMENTS

This project was conducted at the University of British Columbia’s San Rafael Research Aviary in British Columbia, Canada with funds provided by the North American Wildlife Foundation through the Delta Waterfowl Research Station, Manitoba, Canada. We thank Robert Etches for assistance with the manuscript.

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