Observations on Ploidy of Cells and on Reproductive Performance in Parthenogenetic Turkeys

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ABSTRACT

Using flow cytometry, the ploidy levels of parthenogenetic turkeys were quantified from blastodisc stage to adulthood. Eggs were collected from noninseminated hens of the Beltsville Small White flock, known for their high degree of parthenogenesis, and the blastodermal cells from developing embryos were compared with those of embryos produced by hens inseminated with semen from males of the same flock. Erythrocytes of parthenogens from Day 10 of incubation to 27 mo of age were also used for ploidy determination. Sperm and erythrocyte preparations from normal males of the above flock served as haploid and diploid standards, respectively.

In parthenogenetically developing blastoderms, 40.3 ± 14.5% of the cells were haploid and 48.9 ± 11.9% diploid; blastoderms from fertilized eggs had no haploid cells. The haploid cell content of parthenogens declined from the blastodermal stage to adult life, with 1.9 ± 2.3% at 10 d to 20 d of embryonic development, 1.5 ± 1.4% at 21 to 29 d of development, 1.4 ± 2.6% at 4 wk posthatch, and 1.3 ± 1.9% in adulthood, although changes between the 1st mo after hatch and adult stage were not significant. It is possible, therefore, that parthenogenetic embryos with a low proportion of haploid cells could be the ones that survive to Day 10 of development and beyond, whereas those with a higher proportion of haploid cells fail to develop.

The semen volume of male parthenogens was significantly lower than that of normal males, although the concentration of spermatozoa and their fertilizing capacity did not vary significantly between groups, suggesting that the germ cells of these parthenogens are capable of normal meiosis and sperm maturation leading to a normal fertility.

(Key words: parthenogenetic turkey, ploidy, reproduction, parthenogenesis, chromosome)

INTRODUCTION

In turkeys, parthenogenetic development is initiated spontaneously in a large percentage of the eggs (Olsen and Marsden, 1953, 1954; Olsen, 1972; Schom et al., 1982; Savage and Harper, 1986), with some reports indicating as much as 49.3% occurrence (Olsen, 1972), although development is completed only in a small percentage of parthenogens (Olsen, 1975). Early embryonic development is often delayed in parthenogens and, consequently, hatching occurs 2 to 3 d later for parthenogens than for normal embryos derived from inseminated hens (Olsen, 1965). Only 20% of the parthenogens that complete embryonic development survive to produce offspring (Olsen, 1975).

Darcey and Buss (1968) have suggested that parthenogenetic development in the turkey begins in a haploid oocyte, and, at some subsequent developmental stage, the diploid chromosome number is established. The mechanism through which diploidy is attained, however, has yet to be elucidated. Some parthenogens are heterozygous at one or more of the histocompatibility (Poole et al., 1963) and down color (Olsen, 1966) loci, indicating that the diploid number could be the result of union of the haploid egg nucleus and the second polar body or lack of meiosis II. In either scenario, heterozygosity would be created by crossing over during meiosis I. From these observations, it is concluded that parthenogens originate from postmeiotic doubling of ootids.

Although the chromosome make-up of parthenogenetically derived turkey embryos has been reported previously based on selected macrochromosome analysis in squash preparations (Darcey et al., 1971; Harada and Buss, 1981a), the proportions of different ploidy levels during various phases of the bird’s growth from the early embryonic stage to sexually mature adult life have not been established, because the conventional microscopic techniques employed were laborious and their reliability depended on the percentage of cells

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Abbreviation Key: RBC = red blood cells.
undergoing mitosis at the time they were examined. In the present study, we used flow cytometry, which offers a reliable profile of ploidy levels in a large number of cells any time without recourse to inducing cell proliferation in the sample collected. This relatively noninvasive method also allows repeated sampling to test genomic composition without causing undue stress to the birds.

MATERIALS AND METHODS

**Determination of Frequency of Parthenogenesis**

Turkeys from the Beltsville Small White line, selected for high incidence of parthenogenesis (Olsen, 1975), were kindly provided by Hans Abplanalp, University of California, Davis. All birds used in the present study were from this line, which has been designated at the University of Guelph as BSW HiPar. Eggs were collected between 1993 and 1995 from virgin hens or hens that had not been inseminated within the preceding 4 mo and had molted between the last insemination and collection of eggs for this study. Data were collected on 6,730 eggs incubated to determine the proportions of eggs reaching various stages of parthenogenetic development. The eggs were collected three to four times daily, stored for up to 1 wk at 15 C and 75% humidity, and then placed into an incubator at 37.5 C and 50% relative humidity. Eggs were candled between the 7th and 14th d of incubation and eggs of no apparent growth, or those containing dead embryos, were opened and examined for evidence of development. Embryos that were alive on Day 25 were transferred to a hatching compartment and maintained at 36.5 C and 75% relative humidity until they hatched.

**Determination of Ploidy of Blastodermal Cells**

Developing blastoderms were collected and washed as described by Petitte et al. (1990) in PBS-G and stored at −70 C in PBS until analyzed. Fifty-four blastoderms were collected from unincubated eggs from virgin and non-mated hens, and 17 samples were prepared from blastoderms obtained from eggs laid by hens following fertilization with semen from normal males (nonparthenogenetic) of the same stock. Samples of turkey sperm (n = 6) and red blood cells (RBC) (n = 6) from BSW HiPar toms were prepared as haploid and diploid standards, respectively, according to the instructions provided with the CycleTest Plus DNA Reagent Kit. The chromosome make up of cells in each sample was determined by flow cytometry using the Cellfit model on a Becton Dickinson flow cytometer. The proportions of haploid and diploid cells were calculated using the MANL model.

**Determination of Ploidy in Erythrocytes of Developing, Juvenile, and Adult Parthenogens**

Blood was collected from six parthenogenetic embryos between Days 10 and 20 of incubation and from another eight parthenogens between Days 21 and 29 of incubation. Blood was also collected from a different group of five parthenogens within 4 wk after hatching and at 6 mo of age to determine whether the proportion of haploid cells display any shift during sexual maturation. Blood samples during adult stages (20 wk to 27 mo) were also obtained from three other parthenogens, one of which hatched with curled toes, grew very slowly, and died at 20 wk of age; this bird did not exhibit male behavior and never produced semen. Of the two remaining, one developed normally, and exhibited normal male behavior, but produced very little semen and sired only one offspring, whereas the other one developed normally, exhibited male behavior, produced semen, and sired several offspring. The cells were prepared in duplicate and the ploidy levels in each sample were determined by flow cytometry as described above.

**Assessment of Reproductive Performance of Males**

Semen was manually collected from six normal males and four parthenogenetic males housed in adjacent pens. Semen volume was estimated by weight and the concentration of sperm cells was estimated using a hemocytometer. Fertility was determined by inseminating hens from BSW HiPar line with semen from parthenogenetic and normal males of the same line. A total of 82 hens were inseminated during the course of 2 yr (1992 and 1994). Eggs were candled between Day 7 and 10 of incubation and the hatch of poult was recorded.

**Statistical Analysis**

Calculations of means, standard errors, and mean comparisons were conducted using the GLM procedures of SAS® (SAS Institute, 1987). An ANOVA was employed for analyses of data on semen volume and spermatozoa concentration.

RESULTS

**Frequency of Parthenogenesis**

Of the 6,730 infertile eggs incubated over a 3-yr period, 71.3% showed no form of development, whereas 25.8% developed into an unorganized sheet of cells, 1.1% stopped developing at the blood island stage, 1.5% survived to Day 25 of incubation, and 0.3% hatched (Table 1).
**Proportions of Haploid and Diploid Cells in Parthenogenetically Derived Blastoderms, Embryos, Poults, and Adults**

The mean percentage of haploid, diploid, and heteroploid cells in each sample group is shown in Table 2. In parthenogenetic blastoderms, the mean percentage of haploid and diploid cells was 40.3 ± 14.5 and 48.9 ± 11.9%, respectively. Of the 54 parthenogenetic blastoderms analyzed, one had less than 5% haploid cells, three had between 10 and 20%, and the remaining 50 had between 20 and 76% haploid cells (not itemized in the Table). Haploid cells were not observed in blastoderms derived from normal fertilized egg. The proportions of diploid cells in blastoderms of fertilized eggs were significantly greater ($P < 0.05$) than those of parthenogenetic blastoderms. The percentage of haploid cells from the blastoderm stage of parthenogenetic embryos was significantly greater ($P < 0.01$) than those of parthenogenetic embryos collected from all subsequent stages ("10- to 20-d incubation stage" through adulthood) of development (Table 2). Although a trend showing a continued decline in haploid cell concentration was apparent from "10- to 20-d incubation stage" through adulthood (i.e., a decline from 1.85 to 1.25% in haploid cell proportion), there were no statistically significant differences ($P > 0.1$) between these groups (Table 2).

Flow cytometric analysis of erythrocytes taken 5 to 6 mo after the initial evaluation revealed that the proportions of haploid cells were unchanged in five of the six surviving birds (Table 3). However, the proportion of haploid cells in one poult dramatically increased from less than 1 to over 8%, by 6 mo of age; it is not clear whether or not this represents an unusual occurrence or a frequent incident.

**Reproductive Performance of Parthenogenetic Turkeys**

The volume of semen produced by parthenogenetic males was significantly ($P \leq 0.05$) smaller than that of semen produced by normal (nonparthenogenetic) males (Table 4). The concentration of spermatozoa in the semen, however, was not significantly different ($P \geq 0.05$) between parthenogenetic and normal males. The fertilizing capacity of semen from parthenogenetic males is listed in Table 5. Although the number of parthenogenetic males included in this study is rather small, an *ad hoc* comparison of the data (Table 5) indicates that the fertilizing capacity of the semen from parthenogens is not affected.

**DISCUSSION**

Our data indicate that development is initiated in approximately 30% of eggs from virgin and nonmated BSW HiPar hens, following incubation (Table 1). Olsen (1965) reported that 17% of eggs from the original BSW line exhibited parthenogenetic development in 1952 and
that after 13 yr of selective breeding, the rate of parthenogenesis was increased to 45%; in a subsequent study, Olsen (1972) observed as much as 49.3% parthenogenesis. It should be noted here that the use of certain live vaccines in turkeys could enhance the incidence of parthenogenesis in their eggs (Olsen, 1956, Olsen and Buss, 1967); however, no such vaccines were used in our flock. In the present study, some of the embryos in which development was arrested at a very early stage may not have been included because they disintegrated during the interval between embryonic death and examination. The possibility that the estimated 30% parthenogenesis in the present study is conservative is supported by the results of our examination of an additional 343 unincubated eggs on the day of lay, which yielded a rate of 46% parthenogenesis (data not shown).

Our analysis of the distribution of haploid and diploid cells in parthenogenetically developing blastoderms indicates that the proportions of haploid and diploid cells are comparable at the blastodermal stage, although by Day 10 of incubation diploid cells become more abundant. It is interesting to note that the proportions of haploid cells in parthenogens do not change significantly from Day 10 to adult life (Table 2). It appears that the rare parthenogens with small populations of haploid cells on the day of lay might be the ones that are likely to continue development and possibly hatch, because those that survive beyond Day 10 of incubation are predominantly diploid. This observation indicates that developing parthenogens that become predominantly diploid within a few days of incubation, or those that are already predominantly diploid on the day of lay will continue to develop, and may eventually hatch. The majority of developing parthenogens, however, die after a few days of incubation. These embryos probably maintained a substantial proportion of haploid cells, which prevented development to later stages.

Only 3% of incubated eggs contained parthenogenetic embryos that survived beyond the first few days of incubation (Table 1), and these embryos are probably the

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**TABLE 3. Phenotype and proportion of haploid cells in hatched parthenogens**

<table>
<thead>
<tr>
<th>Turkey no.</th>
<th>Description</th>
<th>Age at first analysis</th>
<th>Percentage of haploid cells</th>
<th>Age at second analysis</th>
<th>Percentage of haploid cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4144–4145</td>
<td>Abnormal development; no male behavior; no semen; died at 20 wk</td>
<td>20 wk</td>
<td>3.35</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4201–4141</td>
<td>Male behavior; low semen production; one offspring.</td>
<td>19 mo</td>
<td>0.35</td>
<td>26 mo</td>
<td>0.34</td>
</tr>
<tr>
<td>4127–4128</td>
<td>Male behavior; normal semen production; over 50 offspring.</td>
<td>20 mo</td>
<td>0.01</td>
<td>27 mo</td>
<td>0.02</td>
</tr>
<tr>
<td>261–220</td>
<td>Normal development.</td>
<td>0 to 4 wk</td>
<td>0.10</td>
<td>5 to 6 mo</td>
<td>0.20</td>
</tr>
<tr>
<td>222–276</td>
<td>Normal development.</td>
<td>0 to 4 wk</td>
<td>0.19</td>
<td>5 to 6 mo</td>
<td>0.19</td>
</tr>
<tr>
<td>261–220</td>
<td>Died after 3 d.</td>
<td>0 to 4 wk</td>
<td>6.10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>301–302</td>
<td>Crooked toes but developed normally</td>
<td>0 to 4 wk</td>
<td>0.03</td>
<td>5 to 6 mo</td>
<td>0.03</td>
</tr>
<tr>
<td>221–263</td>
<td>Normal development.</td>
<td>0 to 4 wk</td>
<td>0.73</td>
<td>5 to 6 mo</td>
<td>8.91</td>
</tr>
</tbody>
</table>

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**TABLE 4. Volume of ejaculated semen and concentration of spermatozoa in the ejaculates of normal and parthenogenetic male turkeys from the BSW HiPar line**

<table>
<thead>
<tr>
<th>Turkey no.</th>
<th>Normal males</th>
<th>Parthenogenetic males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume</td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>(mL)</td>
<td>(10^9 cells/mL)</td>
</tr>
<tr>
<td>4110</td>
<td>0.2643</td>
<td>3.5</td>
</tr>
<tr>
<td>4139</td>
<td>0.1654</td>
<td>6.9</td>
</tr>
<tr>
<td>4119</td>
<td>0.1151</td>
<td>5.2</td>
</tr>
<tr>
<td>4148</td>
<td>0.1203</td>
<td>5.9</td>
</tr>
<tr>
<td>4143</td>
<td>0.0530</td>
<td>5.4</td>
</tr>
<tr>
<td>4109</td>
<td>0.1818</td>
<td>7.5</td>
</tr>
<tr>
<td>4127–28</td>
<td>0.0293</td>
<td>5.7 ± 0.57</td>
</tr>
<tr>
<td>301–302</td>
<td>0.0749</td>
<td>5.7 ± 0.57</td>
</tr>
<tr>
<td>222–276</td>
<td>0.0592</td>
<td>4.9</td>
</tr>
<tr>
<td>261–220</td>
<td>0.0620</td>
<td>7.2</td>
</tr>
<tr>
<td>Mean</td>
<td>0.1500 ± 0.0293</td>
<td>5.7 ± 0.57</td>
</tr>
</tbody>
</table>

^1Mean volume significantly (P < 0.05) different from that of normal males.

^2Mean concentration did not vary significantly (P > 0.05) from that of normal males.
small group with low haploid populations on the day of lay. Previous reports suggested that parthenogenetic embryos initiate their development in haploid ova (Olsen, 1975; Harada and Buss, 1981b). The presence of haploid cells in all of the parthenogenetically developing turkey embryos that we have examined provides strong evidence that parthenogenetic development is initiated in the haploid ovum, which subsequently gives rise to a diploid population within the first 24 h of development. If the diploid population is established before many haploid cell divisions occur, then a predominantly diploid blastoderm is created by the time the egg is laid. Such embryos probably have a good chance of continued development and hatching. On the other hand, if a substantial haploid population is established before diploidy is restored, development ceases after a few days.

It may be mentioned here that Fechheimer (1981) reported the occurrence of haploid cell lines among diploid chick embryos derived from normal fertilization. However, we are not aware of similar observations in turkeys. The present investigation provided no evidence for the presence of haploid cells in embryos derived from fertilized eggs.

Examination of chromosomes of parthenogenetic embryos has provided evidence that they begin as haploid eggs and subsequently become diploid embryos (Darcey and Buss, 1968; Darcey et al., 1971; Harada and Buss, 1981b). Haploid and diploid cells were observed in blastoderms from unincubated embryos in approximately equal frequency, although more than 70% of the cells could not be analyzed for technical reasons (Darcey et al., 1971). Substantial proportions of haploid cells were identified in parthenogens between Days 5 and 9 of development using a cytfluorometric technique (DeFord et al., 1979). Haploid, diploid, and polyploid cells were identified in parthenogens up to 2 d of development, although only haploid and diploid cells were found in embryos between 2 and 5 d of development (Harada and Buss, 1981a). In the present study, we have observed that a small population of haploid cells continues to contribute to parthenogens in adult life (Table 2) and that there is a strong inverse association between the proportion of haploid cells and the initiation of organized embryonic development. Most of the parthenogens that have lived to 6 mo of age or produced semen have had less than 1% haploid erythrocytes (Table 3). The only exception to this generalization is bird No. 221-263, in which the proportion of haploid cells increased from 0.7 to 8.9% between hatch and sexual maturity. The increase in the proportion of haploid cells in this poult between 1 and 6 mo indicates that the haploid:diploid ratio can change after hatching.

Although the sperm concentration and fertility in the parthenogens are normal, the mean semen volume of the parthenogens (0.05 mL) is only 30% of that of the normal turkeys (0.15 mL) (Tables 4 and 5). This may be related to the small population of haploid cells in mature parthenogens. A reduced testicular size (Olsen, 1970, 1973), could also be attributed to the low semen volume in parthenogens, as testicular weight and volume are correlated to daily sperm production (Etches, 1996). Although all of the mature parthenogens that have been reared at Guelph have produced semen, the experience at Beltsville indicated that only 20% of mature parthenogenetic toms produced semen (Olsen, 1975) and the fertilizing capacity of semen was estimated to be less than 50% (Olsen, 1965, 1970). Taken together, it would appear that the presence of a small population of haploid spermatogonia reduces semen production in parthenogenetic males, but more conclusive evidence is required to establish such a correlation. Alternatively, Savage and Harper (1986) have reported that a high incidence of parthenogenesis is associated with low semen volume in turkeys. They noted that the high rate of parthenogenesis (28%) in Medium White Turkey line selected for low semen volume and conversely, the low rate (4%) in a line selected for high semen volume, may indicate either an unselected response to genetic selection pressure for decreased semen volume as a means of genome survival. However, the sperm concentration in the parthenogens indicate that spermatogenesis is not seriously compromised and that the spermatozoa are capable of maturation.

The matings recorded in Table 5 include insemination of parthenogenetic males to their dams. Because par-
thenogenetic males are derived entirely from the maternal genome, the equivalent of self-fertilization has been achieved when parthenogenetic males are bred to their mothers. In both experimental biology and in the turkey breeding industry, the opportunity to maintain lines without introducing new genetic variation could have practical value and provide a unique experimental model.

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