A CYTOLOGICAL STUDY OF EARLY CELL POPULATIONS IN DEVELOPING PARTHENOGENETIC BLASTODISCS OF THE TURKEY[1]

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APPROXIMATELY 17% of the eggs laid by non-mated Beltsville Small White (BSW) turkeys showed some type (e.g., membranes, blood, embryos) of development upon incubation (OLSEN and MARSDEN 1953, 1954).

The question arose as to whether the cells in such unfertilized eggs were haploid or diploid. YAO and OLSEN (1955) reported finding diploid chromosome numbers in embryonic tissue of parthenogenetic origin, and hypothesized that chromosome doubling must occur at an early stage in development allowing regular mitotic cell division to proceed with the formation of normal embryonic tissue. POOLE (1959) extended the observations of YAO and OLSEN (1955) to hatched parthenogenetic turkeys (all males). No morphological or numerical differences were found between the chromosome complements of pinfeather cells from normal and parthenogenetic turkey. Finally, SATO and KOSIN (1960) examined the chromosome number of incubated parthenogenetic germ discs and embryos and found that most of the dividing cells contained the diploid chromosome number. Some haploid and polyploid chromosome numbers were also observed by them in the parthenogenetic material. Observations of the gonads of 67 parthenogenetic embryos indicated they were all males (POOLE and OLSEN 1957).

With the knowledge that upon incubation a certain percentage of the eggs laid by virgin turkeys will develop into diploid parthenogens, it is of interest to determine at what point in development the parthenogen attains the diploid chromosome number. The objective of this investigation was to determine if abnormal oogenesis, resulting in an unreduced oocyte, is the basis of the diploid parthenogen. To accomplish this goal blastodiscs from newly laid, unincubated eggs as well as from incubated eggs were selected for study. Embryos derived from matings of turkeys selected over a 12-year period for a higher incidence of parthenogenetic development were chosen to determine if triploids are formed.

MATERIALS AND METHODS

Blastodiscs from newly laid, unincubated parthenogenetically developing eggs from two groups of virgin females (at The Pennsylvania State University), one characterized by a high incidence,

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the other a lower incidence of parthenogenesis, were chosen for chromosome analysis. Also, from the high-incidence line 72-hr turkey embryos from fertilized eggs were studied to determine if triploids were being produced by fertilization of a diploid egg cell. Further, sectioned blastodiscs from unincubated and incubated unfertilized turkey eggs (from the Beltsville station) were examined cytologically. This provided a comparison between sectioned and squashed blastodisc cells.

Embryos from fertilized eggs: Eggs produced from six high-incidence BSW females, after artificial insemination with semen from males of the same strain, were incubated for 72 hr. Squash preparations were made as previously reported (Bloom and Buss 1967; Bloom 1969b).

Squashed blastodiscs from unfertilized eggs: Newly laid eggs from virgin BSW females were incubated for 4–6 hr preceding the following treatment.

a. Colchicine treatment: Upon removal from the incubator, a small window was cut into the side of the egg, the blastodisc located and 0.05 cc of 0.02% colchicine injected into the yolk alongside the blastodisc (Owen 1965; Bloom and Buss 1966; Bloom 1969b). The egg was then replaced in the incubator at 37°C for a 45-minute period.

b. Sodium citrate treatment: After the 45-minute colchicine treatment, the egg was broken out, the albumin removed, and the yolk and blastodisc covered with 0.9% sodium citrate (Owen 1965; Bloom 1969a). Again, the eggs was replaced in the incubator at 37°C for a 45-minute period.

c. Fixation: After 45 min, the sodium citrate was decanted and the yolk and blastodisc covered with acetic-alcohol (1:3) fixative for 24 hr (Owen 1965; Bloom 1969b). The position of the blastodisc on the yolk was marked at this time by inserting two straw splinters into the yolk on opposite sides of the blastodisc.

d. Squashing: After fixation the blastodisc was placed in a well slide containing 45% aceto-orcein stain. The blastodisc was macerated by means of two metal dissecting needles. Using a Pasteur pipet, a suspension of the tissue was transferred to a clean slide, a cover slip placed on top, and the tissue squashed (Bloom and Buss 1967).

Sectioned Blastodiscs From Unfertilized Egg: Sections made previously (Olsen 1965) were re-examined by one of us (S. E. Bloom) to determine numbers of cells, mitotic index and ploidy of cells.

Microscopy: All slides were examined by using either bright field or phase contrast microscopy at magnifications of 400 and 800 times. For purposes of closer examination of cells of particular interest, oil immersion at a magnification of 1800 times was used. Photographs were taken with a Leitz Orthomat camera attached to the Leitz Ortholux microscope.

RESULTS

Embryos From Fertilized Eggs: Thirty embryos, five from each of six mated high-incidence BSW females, were examined. A total of 220 metaphases cells (all diploid) was observed.

Blastodiscs From Unfertilized Eggs: The record of parthenogenesis for the two groups of virgin birds used during this investigation, the high-incidence strain and the lower-incidence F_2 females, is presented in Table 1. The F_2 females which had an average incidence of parthenogenesis of 28% were produced from sib-mating of individuals from an F_1 population. The F_1 population was the result of crosses between females having a 48% incidence of parthenogenesis and males whose sisters exhibited a 4% incidence of parthenogenesis.

Blastodiscs from five high-incidence and six F_2 female turkeys were used for determining the number of interphase nuclei, the total number of mitotic nuclei (Table 1) and the ploidy of metaphase cells as found from karyotypes of the four largest chromosome pairs (Table 2). The major reason for not counting the

TABLE 1

Frequency of mitosis in BSW turkey eggs and its association with the percent of parthenogenesis

Dam number	Percent parthenogenesis	Number of nuclei exhibiting		Percent mitosis	Number of nuclei in which chromosomes were counted
		Interphase	Mitosis		
High-incidence BSW females					
136	48	149	50	34	4
137	38	1269	228	18	166
144	18	278	95	34	29
146	50	1163	193	17	65
146	50	266	29	11	17
F_2 population of BSW females					
68	23	676	63	9	30
70	16	262	59	23	6
72	52	169	80	47	61
78	12	1013	143	14	43
80	21	183	63	34	25
81	18	609	49	8	28

chromosomes in all of the mitotic nuclei observed was the presence of overlapping chromosomes which made identification and counts of the larger chromosomes impossible.

The number of cells, interphase and mitotic, was observed to vary from one blastodisc to another (Table 1). The variation is especially evident with respect to the two blastodiscs from hen #146 (Table 1); one blastodisc had approximately four times as many cells as the other.

1. High-incidence females: Fifty-eight blastodiscs from the eggs of high-incidence virgin BSW turkeys were examined. Of these, 16 were found to contain no cells, and 10 were found to contain only cells with nuclei in the interphase stage. Of the 32 blastodiscs showing parthenogenetic development, it was possible to count the chromosomes in 16; the chromosomes in the remaining 16 could not be counted for various reasons, the major one being excessive scattering of the chromosomes.

The data from the 16 blastodiscs which contained mitotic nuclei that could be scored for chromosome numbers are presented in Table 2. The following cell types were observed: those containing only the haploid (A–Z) number of chromosomes; those containing the diploid (2A–ZZ) number of chromosomes; and those that appeared to be aneuploid or polyploid. Figures 1 and 2 are examples of diploid and haploid nuclei, respectively, observed during this investigation, and Figure 3 presents partial karyotypes of the chromosomes from Figures 1 and 2. All diploid nuclei observed had two Z chromosomes (sex chromosomes) and therefore were male.

For 10 of the 16 blastodiscs from eggs laid by the high-incidence females, the number of mitotic nuclei counted containing the haploid number of chromosomes in each blastodisc was greater than the number of nuclei having the diploid number. The remaining 6 blastodiscs; however, were observed to each have a

TABLE 2

Chromosome counts in parthenogenetic blastodiscs

Dam number		Counted	Number of nuclei Haploid	Diploid	Other
		High-incidence BSW females			
133		27	12	0	15
134		10	2	1	7
136		36	6	1	29
136		4	0	4	..
137		10	4	1	5
137		70	13	5	52
137		166	15	12	139
140		2	2	0	..
144		5	1	4	..
144		29	4	1	24
145		3	2	1	..
146		8	0	3	5
146		22	14	8	..
146		40	2	6	32
146		65	4	13	48
146		17	1	2	14
	Total:	514	82	62	370
	Percent:		16	12	72
		F_2 population of BSW females			
68		30	8	10	12
70		6	2	1	3
72		61	0	3	58
75		52	2	0	50
78		43	3	14	26
80		25	5	7	13
81		18	9	9	..
81		28	3	3	22
	Total:	263	32	47	184
	Percent:		12	18	70

greater number of mitotic nuclei containing the diploid number of chromosomes. Among the 514 cells in which the chromosome number was established, 16%, 12% and 72% were haploid, diploid, or other ploidy (Table 2).

2. F_2 females: Nine blastodiscs from the eggs of an F_2 population of virgin BSW turkeys were examined. Of these, one was found after cytological examination to contain no cells, whereas the remaining 8 were found to be undergoing parthenogenetic development. It was possible to count the chromosomes in all 8 of the developing blastodiscs. For 2 of the 8 blastodiscs, the number of haploid cells exceeded the number of diploid cells. The remaining 6 blastodiscs, however, were observed to have either equal numbers of diploid and haploid cells, or a greater number of cells containing the diploid number of chromosomes. Among the 263 cells in which the chromosome number was established, 12%, 18% and 70% were haploid, diploid or other ploidy (Table 2).

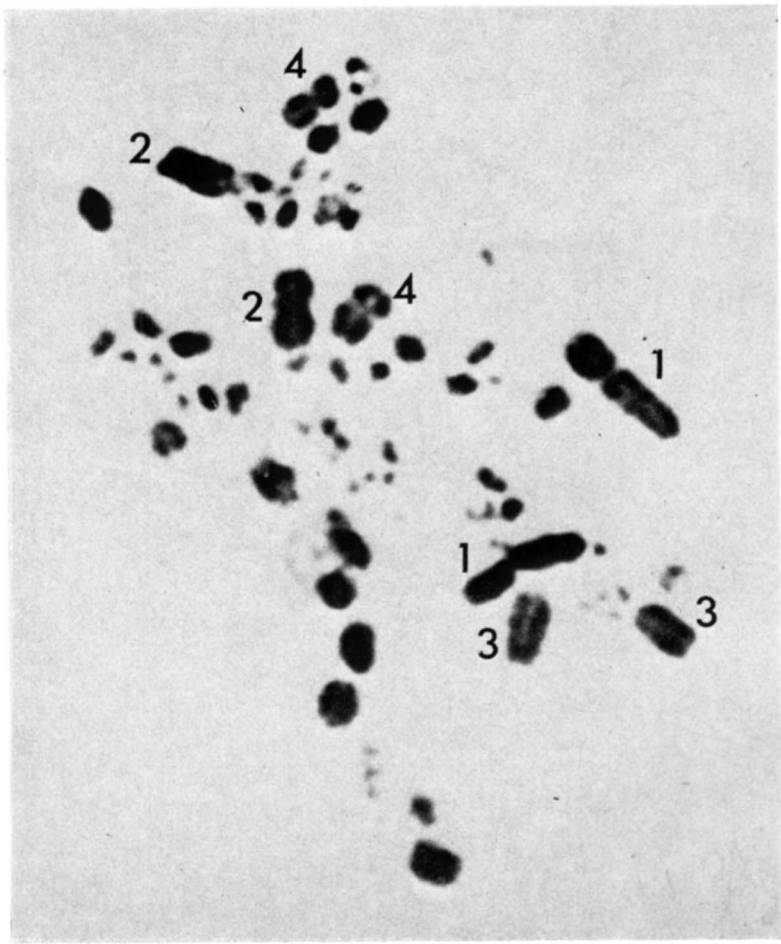

FIGURE 1.—Diploid (2A-ZZ) metaphase from a squashed blastodisc of a newly laid, unincubated parthenogenetically developing turkey egg. 2,900 ×.

The largest number of metaphase cells were classified as "other", i.e., aneuploid, polyploid or artifact (Table 2). This group represents a collection of metaphases that were not readily identifiable as haploid or diploid. Since the production of artifacts was common (due to chromosome scattering) it was not possible to determine the actual level of aneuploidy or polyploidy.

Cells and chromosomes from sectioned blastodiscs (unfertilized): Sectioned blastodiscs from unfertilized turkey eggs at 0 to 3 D.I. (days of incubation) were examined. Cell nuclei, nucleoli, and chromosomes were visible in these sections (Table 3).

Haploid (A–Z) and diploid (2A–ZZ) cells were present in all sections at all stages examined. Polyploid (mostly triploid) cells were sporadically encountered.

The relative number of mitotic cells decreased from 0 to 3 D.I. In the clearest

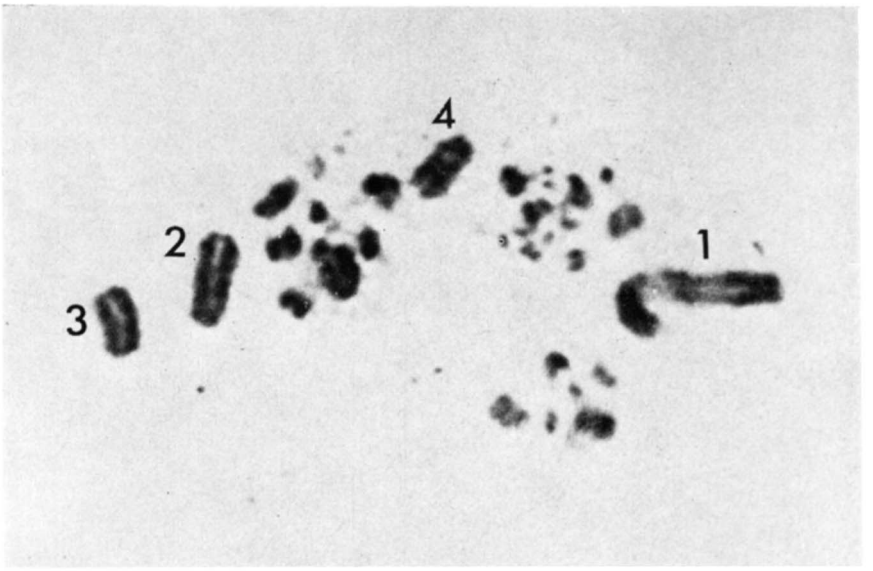

FIGURE 2.—Haploid (A-Z) metaphase from a squashed blastodisc of a newly laid, unincubated parthenogenetically developing turkey egg. 2,900 ×.

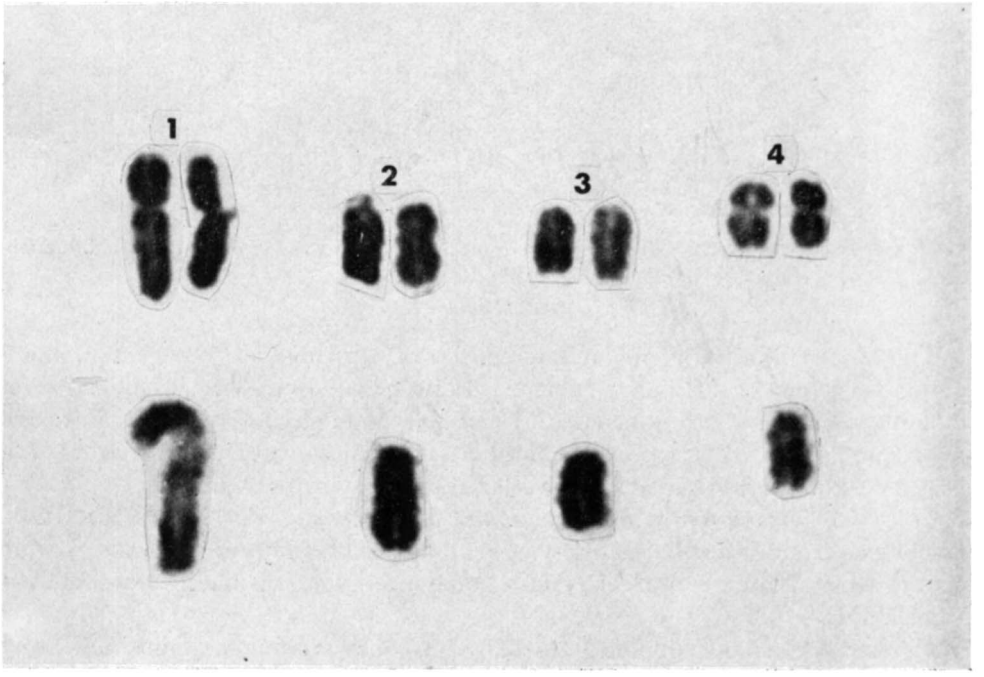

FIGURE 3.—Karyotype of the three largest autosomes and the sex chromosomes from the metaphase cells in Figures 1 and 2.

TABLE 3

The effects of duration of incubation on cell ploidy and the proportion of mitoses in sectioned blastodiscs from unfertilized turkey eggs

Slide number	Days of incubation	Number nuclei counted	Metaphases*			Number mitoses	Percent mitoses
			Haploid	Diploid	Polyploid		
169 C	0	2686	+	+	+	—	—
171 D	0	2300	+	+	+	—	—
171 E	0	2106	+	+	+	—	—
173 C	0	947	+	+	—	—	—
173 E	0	1114	+	+	+	63	5.7
179 E	0	1802	+	+	—	77	4.3
179 G	0	2460	+	+	+	266	10.8
180 A	0	1971	+	+	+	557	28.3
1 D	1	1018	+	+	—	112	11.0
1 F	1	1717	+	+	+	112	6.5
2 C	1	160	+	+	—	21	13.1
4 D	2	5141	+	+	+	163	3.2
5 D	2	3214	+	+	+	104	3.2
24 A	2	395	+	+	—	15	3.8
25 A	2	3953	+	+	—	125	3.2
28 A	3	8678	+	+	—	217	2.5
28 B	3	8643	+	+	—	126	1.5
28 E	3	7460	+	+	+	111	1.5

* A + sign denotes the presence of at least 10 isolated nuclei for the cell type, while a — sign denotes the abscence of a cell type. Each section had several nuclei, and there were 25–39 sections for each blastodisc studied.

section (slide no. 169c, Table 3), the ratio number of haploid metaphases/number of diploid metaphases was 2.4.

DISCUSSION

Embryos From Fertilized Eggs: Observations of the diploid chromosome number in the somatic tissue of embryos produced from the mating of six high-incidence BSW females indicates normal fertilization involving the fusion of male (A–Z) and female (A–Z or A–W) gametes each containing the haploid chromosome number. The six high-incidence females used during this investigation produced, as virgins, eggs showing 49, 65, 58, 45, 51 and 55% parthenogenetic development. After insemination the same six females produced eggs showing 94, 100, 94, 100, 95 and 80% fertility (Buss, unpublished data). The high fertility coupled with the high incidence of parthenogenesis should have insured the detection of any abnormality (e.g., triploidy) among the thirty embryos studied. Olsen (1967) stated that when BSW females of the strain that produces a high incidence of parthenogenesis were mated, 97% of the eggs were fertilized and approximately 90% of the fertilized eggs developed into normal poults. If oogenesis, in females capable of parthenogenetic reproduction, is ab-

normal either due to the suppression of meiosis I or II or reentry of one of the polar bodies then triploid progeny should be observed when these females are mated to normal males.

The mode of diploidization in parthenogenetic eggs of *Drosophila mercatorum* was studied by CARSON, WEI and HIEDERKORN in 1969. More than 90% of the females produced parthenogenetically were found to be isogenic, and it was concluded that these probably arose from post-meiotic doubling of a haploid nucleus. Heterozygosity was found in the remainder of the impaternate offspring which apparently arose through fusion of two haploid meiotic products.

Parthenogenesis in the rock lizard (DAREVSKY 1966) as in the turkey (YAO and OLSEN 1955), produces a diploid parthenogen. However, the origin of the diploid chromosome number in the lizard parthenogen is, according to DAREVSKY and KULIKOVA (1964), nondisjunction at meiosis II. Sexual reproduction involving a female rock lizard capable of parthenogenetic reproduction and a normal bisexual male has been observed to give rise to female progeny with the triploid chromosome number. Results comparable to those obtained by DAREVSKY and KULIKOVA would have been expected in this investigation if the mechanism of diploid parthenogenesis was the same for both the rock lizard and the turkey.

Blastodiscs From Unfertilized Eggs: The presence of haploid as well as as diploid cells in the parthenogenetically developing blastodisc makes questionable the route of diploid parthenogenesis involving suppression of either the first or second meiotic divisions or reentry of either of the polar bodies. The two routes involving suppression or polar body reentry would produce an oocyte containing the diploid chromosome number. Subsequent parthenogenetic development of a diploid oocyte would be expected to give rise to only more diploid cells.

The route of diploid parthenogenesis involving nuclear without cytoplasmic division of the single haploid female pronucleus, following meiosis, would also produce parthenogenetic cells all of which possess the diploid chromosome number. The results of this investigation indicate that it is a haploid oocyte which undergoes parthenogenetic development and that at some subsequent point in development diploid cells are formed. SATO and KOSIN (1960) reported, after examining the blastodiscs of eggs laid by virgin BSW females and incubated for seven days, the presence of haploid, diploid, polyploid and binucleated cells. Diploid nuclear plates were observed, as was the case in this investigation, to have two sex chromosomes (2A–ZZ) indicating again that parthenogenetic development in the turkey produces only male parthenogens. The numbers of each type of cell observed was not indicated; however, diploidy was reported to prevail with the occurrence of frequent polyploid cells. Their observations differ somewhat from the present investigation where in 10 of the 16 squashed blastodiscs from the eggs laid by the high-incidence females, in 2 of the 8 squashed blastodiscs from the eggs laid by the F_2 females, and in a sectioned blastodisc the number of haploid metaphases was observed to be greater than the number of diploid metaphases. This study also contrasted with that of SATO and KOSIN (1960) in that no binucleated cells were observed. The differences observed may be the result

of the seven day incubation period used in the investigation of SATO and KOSIN as compared to shorter periods (0 to 6 hr) of incubation of the blastodiscs used in this investigation.

Observation of mitotic nuclei containing the haploid and diploid number of chromosomes in the blastodiscs of eggs from the high-incidence and the F_2 females (Table 2) suggests that with respect to the cell population, there is little difference between the two lines. For 4 of the 8 blastodiscs from the eggs laid by the F_2 females, the number of mitotic nuclei in each containing the diploid number of chromosomes was greater than the number of nuclei containing the haploid number. This indicates that it is not the inability to attain diploidy early in development that causes the low incidence of observable parthenogenetic development characteristic of the F_2 females. The basis for the observed difference between the high and low incidence females has not been determined.

The origin of the aneuploid and polyploid nuclei observed during this investigation remains unknown. It is possible that the small aneuploid nuclei observed during this investigation, were once part of an intact nucleus which was subject to extensive chromosome scattering and are therefore artifacts produced by the squash technique. Another possibility is that they represent a cell type characteristic of parthenogenetic development. The several large polyploid nuclei observed in the blastodiscs may be characteristic of parthenogenetic development and identical to the polyploid cells identified in the incubated material of SATO and KOSIN (1960).

OLSEN (1967) pointed out that fusion of two haploid cells sometime after the first mitotic division, or a nuclear without a cytoplasmic division would produce a completely homozygous parthenogen. OLSEN (1966) mated four (three bronze, *CC*, and a white, *cc*) parthenogens from heterozygous bronze, *Cc*, hens (Beltsville Small White × Bronze) with Beltsville Small White, *cc*, females. The white parthenogen was observed to produce progeny all of which were white. Two of the bronze parthenogens produced only bronze progeny; however, one of the bronze parthenogens produced approximately equal numbers of both bronze and white poults. OLSEN suggested the observed heterozygous parthenogen may have resulted from a crossover between the centromere and the locus for color in meiosis I. In addition to crossing over, failure of the second meiotic division must be invoked to explain the diploid chromosome number of the heterozygous parthenogen; however, these events may have occurred in this one case. The data in Table 2 show that for the 777 nuclei observed in the 24 blastodiscs, one of the two blastodiscs from dam 136 had only diploid nuclei; but only four nuclei were found in this blastodisc. This exceptional blastodisc, with only four diploid nuclei observed, may have been the result of chance. Observation of haploid cells in the parthenogenetic blastodiscs and only diploid cells in embryos from mated turkeys indicates that meiosis is normal in the production of turkey parthenogens.

BLOOM (1969b, 1970) has demonstrated haploidy in chicken embryos. These embryos are mosaics containing mainly haploid (A–Z) and some diploid (2A–

ZZ) and triploid (3A–ZZZ) cells. In this instance, only mated hens produced haploid embryos. Therefore, the spermatozoon acted to stimulate development and in a manner suggesting gynogenesis.

Thus, studies of both turkeys and chickens suggest that haploid gametes, when properly stimulated, can undergo mitosis to form populations of haploid (A–Z) cells and that these cells may then participate in diploid and polyploid cell formation. Embryos may then be organized and, in turkeys, diploid (2A–ZZ) embryos can hatch and mature normally.

SUMMARY

The possibility that abnormal oogenesis, resulting in a diploid unreduced oocyte, is the origin of the diploid parthenogen was investigated. The chromosome number of 30 embryos, 5 from each of 6 mated high-incidence females, was examined. The fact that only diploid embryos were observed suggests that normal chromosome reduction occurs during oogenesis in turkeys capable of parthenogenetic reproduction —The ploidy of cells produced early in parthenogenetic development was determined. Blastodiscs from unincubated eggs of virgin BSW turkeys (having approximately 45% incidence of parthenogenesis) and blastodiscs from unincubated eggs of F_2 virgin BSW turkeys (having approximately 28% incidence of parthenogenesis) were cytologically examined using a squash technique and sectioning. Three cell types were observed: those having the haploid (A–Z) number of chromosomes; those having the diploid (2A–ZZ) number of chromosomes; and those that could be classified only as aneuploid or polyploid.—For 10 of the 16 blastodiscs from eggs laid by the high-incidence females, the number of haploid cells was greater than the number of diploid cells. The remaining 6 blastodiscs each had a greater number of diploid cells.—The results of this investigation indicate that parthenogenetic development begins in a haploid oocyte, and that at some subsequent point in development the diploid chromosome number is restored to produce the observed diploid parthenogen.

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