

Nine Year Summary of Parthenogenesis in Turkeys.* (26082)

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Experiments at this station during the past 9 years have demonstrated, repeatedly, that unfertilized eggs of turkeys can develop to varying degrees upon being incubated. The original observations were made in 1952. Subsequent yearly tests of eggs from selected virgin turkey females have fully confirmed earlier observations. Some of these data have been published(1,2,3,4); but the major portion has not; hence it is desirable that a complete summary be presented.

Confirmation of the occurrence of parthenogenesis in turkey eggs has come from other laboratories. At Oregon State College, Parker noted in 1958 an unorganized type of growth in unfertilized Beltsville Small White turkey eggs following a 14-day period of incubation (personal communication). Kosin, Sato and Nagra, at State College of Washington observed parthenogenetic development in unfertilized eggs from Broad Breasted Bronze, Washington State White, and Beltsville Small White turkeys. These investigators reported on 46 embryos, including one surviving to time of hatch, at the 11th World's Poultry Congress, Mexico City, 1958. Cytological observations based on parthenogenetic tissues were published by Kosin and Nagra(5), Sato and Kosin(6), and Kosin and Sato(7). Their studies, as well as our own(8,9), established diploidy in the somatic cells of parthenogenetic tissues.

Material and methods. Each year, certain Beltsville Small White females which as virgins had demonstrated their ability to produce eggs showing a high incidence of parthenogenesis, were used as breeders. These selected females were mated to males from

families in which the daughters also had demonstrated their ability to produce eggs exhibiting a high incidence of parthenogenesis.

The poults hatched for replacement stock each year were pedigree banded, brooded in batteries until 6 weeks of age, and then transferred to a brooder house. At ages varying from 4-12 weeks, they were separated from their immature male pen mates. Sex identification was accomplished by performing a laparotomy and observing the gonads. From the time of sex identification until maturity, the virgin females were reared in wire enclosed pens to insure against any possible contact with sexually mature males. At the age of 30-36 weeks, the females were transferred to laying houses. Some birds were placed in unheated pens within a large turkey house, others in individual wire cages within a heated building. All birds received the same standard laying mash. Electric lights, used during early morning hours to supplement natural daylight, maintained a 14 hour photoperiod.

Eggs were gathered from trapnests and wire cages, 3 to 4 times daily, and as collected were placed in an incubator at a temperature of 99.5°F and a relative humidity of 57%. In 1952, the eggs tested were given an initial 7-day period of incubation before being candled but in later tests this period of incubation was lengthened to 9-10 days. Eggs in which live embryos could be detected by candling were replaced in the incubator. Those eggs showing growth of membranes only, or no development, were removed, broken, and the discs of each reexamined. Only embryonic growth that could be detected macroscopically was listed as parthenogenetic. All questionable cases and eggs showing no discernible development of the disc were classed as non-parthenogenetic.

Eggs showing parthenogenetic development were classified into 3 categories; (1) those in which the development consisted solely of

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TABLE I. Incidence of Parthenogenetic Development in Unfertilized Eggs of Beltsville Small White Turkeys.

Year	No. of hens	No. eggs tested	—No. and % of eggs found to contain—							
			Membranes only		Blood and membranes		Embryos		All categories of parthenogenesis	
			No.	%	No.	%	No.	%	No.	%
1952	29	934	156	16.7	0		2	.2	158	16.9
1953	23	1463	191	13.1	12	.8	3	.2	206	14.1
1954	103	5930	1198	20.2	71	1.2	44	.74	1313	22.1
1955	110	5757	1251	21.7	161	2.8	84	1.4	1496	26.0
1956	128	6003	1381	23.0	198	3.3	251	4.2	1830	30.5
1957	181	8608	2293	26.6	490	5.7	360	4.2	3143	36.5
1958	214	8045	2060	25.6	492	6.1	722	9.0	3274	40.7
1959	78	2926	718	24.5	153	5.3	342	11.7	1213	41.5
1960	60	2815	720	25.6	123	4.4	277	9.8	1120	39.8
Totals & avg	926	42,481	9968	23.5	1700	4.0	2084	4.9	13,752	32.4

* Percentages are on basis of total eggs tested.

growth of the extraembryonic membranes: (2) those in which differentiation had proceeded to the extent that blood islands or blood vessels were clearly visible by candling or by macroscopic examination and (3) those in which embryos were identifiable on gross examination. Embryos were further classified as to age in terms of equivalent development of the normal turkey embryo. Number of embryos surviving to hatching and those helped from the shell were also listed.

Results and discussion. Results based on examination of more than 42,000 eggs from virgin Beltsville Small White turkeys are summarized in Table I. These data show a steady increase in overall incidence of parthenogenesis, with the most marked increases coming in the categories "blood and membranes" and "embryos." In 1953, 0.8% of all eggs examined were classified in the cate-

gory of "blood and membranes," while in 1958 6.1% were so classified, representing a 7 fold increase. In 1952, embryos were found in 0.2% of all eggs tested, whereas in 1958 the proportion was 11.7%, a 57-fold increase over that observed in eggs of unselected hens used as foundation stock. All embryos and poults in which sex has been determined either by gross(3) or by cytological(10) examination have been male.

Data presented in Table II give number of embryos and poults encountered each year. Also shown are numbers and percentages of embryos that attained the size of normal 2-10, 11-20, and 21-28-day turkey embryos. Listed separately are numbers of parthenogenetic embryos surviving to hatching; most of these were helped from the shell. A definite increase in average age attained by parthenogenetic embryos since 1952 is clearly seen. The increased embryonic viability is

TABLE II. Number and Percentage of Parthenogenetic Embryos Encountered Each Year and Size Each Attained in Relation to That of a Normal Turkey Embryo.

Year	Total No. embryos	Stage of development (days)							
		2-10		11-20		21-28		Hatched	
		No.	%	No.	%	No.	%	No.	%
1952	2	2	100.0						
1953	3	3	100.0						
1954	44	37	84.1	2	4.5	4	9.0	1	2.3
1955	83	70	84.3	5	6.1	8	9.6	0	.0
1956	252	166	65.9	19	7.5	59	23.4	8	3.2
1957	360	249	69.2	28	7.8	71	19.7	12	3.3
1958	721	492	68.3	75	10.4	134	18.5	20	2.8
1959	342	209	61.1	36	10.5	85	24.9	12	3.5
1960	277	155	56.0	20	7.2	87	31.4	15	5.4
Totals & avg	2084	1384	66.4	185	8.9	448	21.5	68	3.3

especially pronounced in the relative number of embryos surviving for 11 days or longer. For example, in 1954 4 of 44 or 9.0% of the embryos attained a size equivalent to that of 21-28-day normal turkey embryos, whereas in 1960 87 of 277 embryos or 31.4% were listed in this age category. Increased viability of parthenogenetic embryos is also reflected in the number of embryos completing their incubation period. Since 1956, 67 poults have been hatched, many of which lived for some time, a few surviving to maturity. Viable spermatozoa have been observed in semen samples obtained from each of 3 parthenogenetic males and offspring have been sired by one male, Olsen(11). In 1960, 15 poults were hatched, 4 of which are still alive, ranging in age from 71 to 136 days. These evidences of increased embryonic viability can be attributed in large part to the selective breeding program initiated in 1954.

Summary. A breeding program for increasing parthenogenetic development, based on the progeny test method, was initiated in 1954. More than 42,000 eggs from 926 Beltsville Small White virgin turkey hens were involved in this 9-year study. A marked increase in incidence of parthenogenesis, par-

ticularly of the more highly organized type, has resulted. Parthenogenetic development was observed in 16.7% of the eggs tested in 1952, in 41.7% in 1959. In 1952, embryos were encountered in 0.2% of all eggs whereas in 1959 11.7% of the eggs were so classified. Of 44 embryos found in 1954, 4 or 9% survived until 21-28 days of incubation; in 1960, 87 of 277 or 31.4% did so. Since 1956 a total of 67 embryos, all males, have survived to hatching, a few reaching maturity. Three males produced spermatozoa and one sired offspring.

1. Olsen, M. W., Marsden, S. J., *Proc. Soc. Exp. Biol. and Med.*, 1953, v82, 638.
2. ———, *J. Exp. Zool.*, 1954, v26, 337.
3. ———, *Science*, 1954, v120, 545.
4. ———, *Poultry Sci.*, 1956, v35, 674.
5. Kosin, I. L., Nagra, H., *Proc. Soc. Exp. Biol. and Med.*, 1956, v93, 605.
6. Sato, I., Kosin, I. L., *Proc. 10th Internat. Congr. of Genetics*, 1958, v2, 249.
7. Kosin, I. L., Sato, I., *Jour. of Morphology*, 1960, v103, 263-276.
8. Yao, T. S., Olsen, M. W., *J. Heredity*, 1955, v46, 133.
9. Poole, H. K., *ibid.*, 1959, v50, 150.
10. Poole, H. K., Olsen, M. W., *ibid.*, 1957, v48, 217.
11. Olsen, M. W., *Science*, 1960, v132 (in press).

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Suppression of Human Choriocarcinoma Maintained in the Hamster Cheek-Pouch by Extracts and Alkaloids of *Vinca rosea*. (26083)

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Noble, Beer and Cutts have reported their pioneer studies on the chemical and biological properties of crude extracts of *Vinca rosea*,* and of the alkaloid, Vincalukoblastine, initially isolated by them from these crude plant extracts(1,2). They noted the marked marrow depressant action of these preparations

in rats and described an interference with estrogen-induced uterine growth in the immature rat as well as carcinostatic effects produced by certain of the active plant fractions. The marrow-depressant, carcinostatic, and uterus-inhibiting properties appeared to resemble the known biological effects of such folic acid antagonists as Methotrexate (4-amino - N10 - methyl - pteroylglutamic acid). Since we had previously observed marked regression of choriocarcinoma in women following intensive Methotrexate therapy it seemed

* The extracts of *Vinca rosea* as well as the specific alkaloids derived therefrom were kindly furnished by Dr. Irving Johnson and Dr. G. H. Svoboda, Eli Lilly Co. Research Labs., Indianapolis, Ind.