VOLUME 58 APRIL 1962 NUMBER 1

THE OCCURRENCE AND POSSIBLE SIGNIFICANCE OF PARTHENOGENESIS IN EGGS OF MATED TURKEYS

M. W. OLSEN

*United States Department of Agriculture**

(Received, April 1960)

Heavy financial losses are experienced each year by many producers of turkey hatching eggs due to low hatchability. Of chief concern is the high incidence of infertile eggs, especially that occurring during the latter part of the regular hatching season. While some factors contributing to infertility are recognized and can be controlled, others of more subtle nature have not been studied.

With the discovery of parthenogenesis in unfertilized turkey eggs in 1952 (Olsen and Marsden, 1953), studies were initiated to determine if this phenomenon was related to infertility. Before this question could be fully resolved, however, it was necessary to establish (1) that parthenogenetic development occurs in unfertilized eggs of mated turkeys, and (2) that parthenogenesis is initiated at a stage of egg formation when it might interfere with the successful union of the male and female pronuclei.

Only circumstantial evidence has been presented thus far to show that parthenogenetic development occurs in unfertilized eggs of mated birds (Olsen and Marsden, 1954a). Turkey eggs laid by mated birds were incubated for 24-72 hours and then segregated by candling into two classes, fertilized and unfertilized. The unfertilized eggs were then given further incubation. Among 675 eggs which appeared clear before the candler at 72 hours of incubation, 151 or 18·2 percent upon further incubation developed embryonic tissue but without embryos. Olsen and Marsden suggested that this unorganized development, occurring in apparently nonfertile eggs, was of parthenogenetic origin, a view not fully shared by Kosin (1958).

The resolution of this question, as was pointed out by Kosin (1958), is 'predicated on finding recognizable differences between fertilized and nonfertilized, but developing germ discs in the eggs of mated hens.' One method of approach would be to mate virgin recessive white turkey females, selected for a high incidence of advanced parthenogenetic embryos, to males of a colored variety. Thus, a genetic down color marker would serve to establish parentage of advanced parthenogenetic embryos and poults. Furthermore, since only males are produced from unfertilized turkey eggs (Poole and Olsen, 1957), it is important that the sex of the embryos of poults in question be recorded. Data of this nature were collected, a portion of which dealt with chicken-turkey hybrids and were presented in another paper (Olsen, 1960).

*Poultry Research Branch, Animal Husbandry Research Division, Agricultural Research Center, ARS, Beltsville, Maryland.

Materials and Methods

One hundred and sixty-four Beltsville Small White turkey hens, *Meleagris gallapavo*, representing a strain of birds which had been selected for the past four generations for a high incidence of parthenogenesis, were used exclusively in these tests. Seventy-seven of these hens were virgins; the other 93, subsequently referred to as previously mated, had been with males 8-10 months earlier.

Seventy-one virgins and 77 previously mated hens were inseminated at intervals of approximately ten days with untreated semen from Dark Cornish chicken males, *Gallus domesticus*. Sixteen other previously mated turkey hens were inseminated with semen from New Jersey Buff turkey males. These males had been tested previously and found to be homozygous for plumage color. Thus, a color marker served to establish parentage of embryos 15 days or older.

To insure that some eggs were not fertilized, the New Jersey Buff turkey semen was chilled at 50°F for one hour before being used. This treatment had been shown earlier to be effective in partially inactivating the semen (Burrows and Quinn, 1939).

The procedures used were described by Olsen and Marsden (1954b), and Olsen (1956). Eggs were placed in the incubator as collected at a temperature of 99·5°F and a relative humidity of 57 percent. All eggs were incubated 9-10 days before being candled. Those in which live embryos could be detected were returned to the incubator, while clear eggs and those showing only growth of membranes were broken out and examined more critically for evidence of development. Only embryonic growth that could be detected macroscopically was considered, and all questionable cases were classed as nonfertilized eggs.

Each egg was classified according to the stage of development into one of three categories: (1) embryonic membranes; (2) embryonic membranes with blood; (3) embryos. Only embryos that could be detected macroscopically were listed in the last category. The color of down and sex of all embryos 15 days or older (advanced embryos) was recorded.

Results

Data summarized in Table 1 show the degree of development encountered in turkey eggs prior to and following time of insemination with semen of Dark Cornish males. Listed separately are the numbers of embryos and ages to which they attained in relation to normal turkey embryos.

Embryos were found in 6·9 percent of the eggs of virgins and in 5·3 percent of those of previously mated hens prior to the time of insemination. This is in contrast with 20·9 percent and 11·7 percent respectively in eggs laid by the same hens following insemination.

A total of 127 embryos was encountered in eggs of virgins following insemination, 54 of which attained an age at which down color served to establish parentage. Of the 54 advanced embryos, including twelve poults which were helped from the shell, 49

Table 1. *Incubation record of eggs laid by young virgin and previously mated two and three year old Beltsville Small White turkey hens before and after insemination with semen of Dark Cornish chicken males*

	Virgin				Previously Mated			
	Before Ins.		After Ins.		Before Ins.		After Ins.	
	No.	%	No.	%	No.	%	No.	%
Total number hens	71	..	71	..	77	..	77	..
Total number eggs	2814	..	609	..	1731	..	997	..
Eggs showing development	1042	37·0	373	61·2	665	38·4	514	51·6
Membranes without blood	699	24·8	159	26·1	459	26·5	297	29·8
Membranes with blood	148	5·3	87	14·3	115	6·6	101	10·1
Embryos	195	6·9	127	20·9	91	5·3	116	11·7
Ages attained by embryos								
2-7 days development	137	70·3	66	52·0	53	58·2	51	44·0
8-14 days development	18	9·2	7	5·5	8	8·8	14	12·1
15-21 days development	11	5·6	11	8·7	5	5·5	9	7·8
22-28 days development	23	11·8	31	24·4	20	22·0	30	25·9
Hatched	6	3·1	12	9·4	5	5·5	12	10·2
Total number embryos	195	100·0	127	100·0	91	100·0	116	100·0

bore the color markings of Dark Cornish chicks. The remaining five embryos, four of which died in the shell and one of which hatched, carried white down. These colorless individuals were males of parthenogenetic origin.

The previously mated hens, after insemination with Dark Cornish semen, produced 997 eggs, 514 or 51·6 percent of which showed some degree of development. One-hundred-sixteen embryos were observed and of these 51 attained an age at which down color established parentage. Thirty-six of the 51 embryos carried color markings of Dark Cornish chicks. Fifteen embryos, including two poults which were helped from the shell on the 29th day of incubation, had white down. All of the white individuals were males of parthenogenetic origin.

Virgin and previously mated hens combined following insemination with Dark Cornish semen produced 243 embryos, 105 of which were 15 days or older. These included 24 poults which were helped from the shell and lived for a few hours or longer. While 85 of the 105 embryos carried the color markings of Dark Cornish chicks, 20 individuals, including three listed as having hatched, were found with white down. Thus, approximately 20 percent of all advanced embryos encountered in eggs from this highly selected strain of turkeys, following insemination with semen from Dark Cornish chickens, originated from unfertilized eggs.

Evidence of parthenogenesis was also found in eggs laid by 16 selected Beltsville Small White turkey hens following insemination with partially inactivated New Jersey Buff turkey semen. A total of 124 eggs were produced, 61 or 49·2 percent of which showed some degree of development upon being incubated. Thirty eggs contained embryos, 26 of which attained a size at which down color served to identify parentage. Twenty-three of the 26 embryos survived to hatching, all poults with the exception of one being dark brown, thus indicating that they were sired by the New Jersey Buff male. One white male poult was helped from the shell and lived for 12 weeks. Two white male embryos found in unhatched eggs had attained a size equal to that of normal turkey embryos of 26-27 days of incubation.

Discussion

These data show that on rare occasions it is possible to obtain a parthenogenetic turkey embryo or poult after hens have been inseminated. Since only diploid parthenogenetic males have been encountered, this means that the haploid female pronucleus is doubled. As was pointed out by Beatty (1957) diploid parthenogenesis may be the result of the suppression or re-entry of either the first or second polar bodies. It may also be brought about, according to Beatty, as a result of a nuclear division without a corresponding cytoplasmic division. In discussing diploidy in parthenogenetic turkeys, Poole (1959) assumes the XX—XO sex chromosome relationship in birds and the lethality of a OO sex chromosome relationship corresponding to the YY lethal in Drosophila (Stalker, 1954). He concludes that 'these are the only cytological mechanisms which might explain diploidy in parthenogenetic turkeys.' Since re-entry of the first polar body should theoretically give rise to some females, this mechanism can probably be discounted as the reason for the doubling of the chromosome number. Diploidy probably results therefore from the suppression, or re-entry, of a second polar body or a nuclear division without a cytoplasmic division.

As was noted earlier, approximately 20 percent of the 105 advanced embryos encountered in eggs of a parthenogenetic strain of Beltsville Small White turkeys, following insemination with Dark Cornish semen, were white males. This means that an ovum from a highly selected turkey hen, if not fertilized, may develop parthenogenetically even to the point of producing a live poult. These data support the contention of Olsen and Marsden (1954a) that parthenogenetic development may occur, although generally unnoticed, in eggs of mated flocks of turkeys.

It should be emphasized, however, that the turkey hens used in this experiment represented a strain selected for a high incidence of advanced parthenogenesis. It should also be emphasized that the matings were highly artificial; chicken sperm, or partially inactivated turkey sperm, could scarcely be expected to equal the fertilizing capacity of normal turkey sperm. Under these restricted conditions, it is not surprising that some parthenogenetic development occurred in selected females; the relatively high proportion of eggs not fertilized by chicken sperm or by partially inactivated turkey sperm presumably behaved as would have a similar proportion of eggs in selected turkeys not subjected to either treatment. Some of these hens, as virgins, produced a considerable number of eggs which contained parthenogenetic embryos (Table I). On the other hand, if unselected hens had been used, which as virgins did not possess the ability to produce parthenogenetic embryos, the results would have been quite different. This was clearly demonstrated in data given in a previous publication, where 24 unselected Beltsville Small White hens were inseminated first with semen of Dark Cornish chickens and later with semen from Rhode Island Red chicken males. Although 66 embryos developed in eggs of the unselected females following insemination, each advanced embryo carried the color of the chicken males used.

Turkey hens in commercial flocks, being unselected for parthenogenesis, would only rarely produce unfertilized eggs in which embryos would develop. Therefore, even if a commercial flock of unselected recessive white turkeys were mated to colored males, it would be extremely rare indeed to encounter a white embryo or poult. This would be even more improbable in instances where eggs were highly fertile.

These data, however, do not contradict the evidence of Olsen and Marsden (1954a), in which an unorganized type of development (presumed to be parthenogenetic) was found in presumably non-fertile eggs from an unselected but mated flock of turkeys. Unpublished data from this station show that unselected virgin Beltsville Small White females regularly produce eggs, 16-20 percent of which, upon being incubated, undergo some degree of parthenogenetic development. This development, however, is generally abnormal and rarely reaches a degree of organization where either blood or embryo formation occurs. This means that any parthenogenetic development occurring in unfertilized eggs following insemination with semen of colored males would be of a type grossly indistinguishable from that taking place in fertilized eggs in which early embryonic mortality had occurred.

Of further interest is the fact that parthenogenetic development is initiated about or possibly after the time of ovulation. Furthermore, the incidence of parthenogenetic cleavage is high, nucleated cells having been found at this station in as high as 90 percent of newly-laid eggs of unselected, virgin, Beltsville Small White turkey females, (Haney and Olsen, 1958). Kosin and Nagra (1956) found nucleated cells in more than 80 percent of the blastodiscs of newly-laid eggs of unselected virgin Broad Breasted Bronze turkeys. Moreover, it has been demonstrated by Yao and Olsen (1955), Sato and Kosin (1958), and Poole (1959) that a doubling of chromosomes occurs in the type of parthenogenesis encountered in turkey eggs. It is conceivable that in some instances the doubling of the chromosomes may occur prior to sperm penetration. If such a situation should arise there is reason to expect that the spermatozoon would be rejected by the already reconstituted nucleus, or at least would be ineffective in bringing about a true fertilization (Blount, 1909). Under such circumstances, one would expect not only a high but an early peak of mortality, particularly during the 24 or more hours that ova are within the oviduct of the hen. Since no development would occur in such eggs after they were placed in the incubator, they would be looked upon, for all practical purposes, as being infertile.

SUMMARY

A study, involving a genetic color marker, was conducted to establish whether parthenogenesis occurs in unfertilized eggs of mated turkeys. One hundred and forty-eight Beltsville Small White turkey hens, representing a strain selected for a high incidence of advanced parthenogenetic development, were inseminated with semen from

Dark Cornish chickens. Sixteen other selected turkey hens were inseminated with partially inactivated semen from homozygous New Jersey Buff turkey males. Plumage color of both Dark Cornish chickens and New Jersey Buff turkey males is dominant to the recessive white of the Beltsville Small White turkey hens.

Following insemination of turkey hens with untreated semen from Dark Cornish chickens, 105 embryos, 15 days of age or older, were encountered. This included 17 embryos and three poults, all being males with white down, indicating that they had developed from unfertilized eggs.

Twenty-two brown poults, one colorless male poult and two colorless male embryos, were obtained from 124 turkey eggs following insemination of Beltsville Small White turkey hens with partially inactivated semen from homozygous New Jersey Buff males. The colorless poult was helped from the shell and survived for 12 weeks. The two colorless embryos attained the size of a normal 26-27 day turkey embryo. The possible significance of parthenogenesis is discussed in relation to infertility in mated flocks of turkeys.

LITERATURE CITED

1. BEATTY, R. A. (1957). *Parthenogenesis and polyploidy in mammalian development.* University Press, Cambridge.
2. BLOUNT, M. (1909). The early development of the pigeon's egg with especial reference to polyspermy and the origin of the periblast nuclei. *Jour. Morph.*, **20,** 1–64.
3. BURROWS, W. H. AND QUINN, J. P. (1939). Artificial insemination of chickens and turkeys. *Proc. Seventh World's Poultry Congress and Exposition,* Cleveland, Ohio. Pages 82–84.
4. HANEY, B. M. AND OLSEN, M. W. (1958). Parthenogenesis in premature and newly laid turkey eggs. *Jour. Exp. Zool.*, **139,** 469–478.
5. KOSIN, I. L. (1958). Time-lag in identification of fertility in Broad Breasted Bronze eggs and its relation to hatchability and possibly parthenogenesis. *Poultry Sci.*, **37,** 1150–1153.
6. KOSIN, I. L. AND NAGRA, HELEN (1956). Frequency of abortive parthenogenesis in domestic turkey. *Proc. Soc. Exp. Biol. and Med.*, **93,** 605–608.
7. OLSEN, M. W. (1960). Turkey Chicken Hybrids. *Jour. Heredity,* **51,** 69-73.
8. OLSEN, M. W. (1956). Fowl pox vaccine associated with parthenogenesis in chicken and turkey eggs. *Science,* **124,** 1078–1079.
9. OLSEN, M. W. AND MARSDEN, S. J. (1953). Embryonic development in turkey eggs laid 60-224 days following removal of males. *Proc. Soc. Exp. Biol. and Med.*, **82,** 638–641.
10. OLSEN, M. W. AND MARSDEN, S. J. (1954a). Mortality among turkey embryos in relation to rate of embryonic development. *Poultry Sci.*, **33,** 1146–1151.
11. OLSEN, M. W. AND MARSDEN, S. J. (1954b). Development of unfertilized turkey eggs. *Jour. Exp. Zool.*, **126,** 337–348.
12. POOLE, H. K. (1959). Mitotic chromosomes of parthenogenetic and normal turkeys. *Jour. Heredity,* **50,** 150–154.
13. POOLE, H. K. AND OLSEN, M. W. (1957). The sex of parthenogenetic turkey embryos. *Jour. Heredity,* **48,** 217–218.
14. SATO, I. AND KOSIN, I. L. (1958). Chromosome complements in parthenogenetic turkey embryos (abstract). *Proc. Tenth International Congress of Genetics,* **2,** 249–250.
15. STALKER, H. P. (1954). Parthenogenesis in Drosophila. *Genetics,* **39,** 4–21.
16. YAO, T. S. AND OLSEN, M. W. (1955). Microscopic observations of parthenogenetic embryonic tissues from virgin turkey eggs. *Jour. Heredity,* **46,** 133–134.