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Effects of Prior Semen Injections and Weekly Inseminations on Hybridization of Chickens and Turkeys. (31975)

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It is now generally accepted that in attempts to cross chickens with turkeys the chicken ♂ × turkey ♀ combination produces greater fertility and embryonic viability than the reciprocal(1). Based on this, and the fact that turkey semen normally has a lower anti-chicken-erythrocyte agglutinin titre than the reciprocal, Ryle(1) postulated that serological (immunological) factors might play some part in inhibiting cross-fertilization or impairing hybrid embryonic viability.

Olsen(2) reported that the chances for successful hybridization of these species are increased when the cross is made with parental strains exhibiting a tendency to reproduce parthenogenetically. His data (unpublished) also indicated that in spite of repeated artificial inseminations hybrid fertility and embryonic development might not have persisted more than 3 weeks following the first insemination.

The data reported here are from 2 experiments performed to explore the following possibilities. 1) Hybrid fertility and embryonic development in turkey eggs might be influenced by prior intraperitoneal injections of hens with semen from the same chicken males by whom they were to be subsequently inseminated. 2) Hybridization, after initial success, might fail regardless of repeated inseminations.

Material and methods. The virgin turkey hens in these experiments were from a Beltsville Small White flock, 16-20% of whose incubated eggs exhibit parthenogenesis. Such development is limited almost exclusively to growth of undifferentiated embryonic membranes(3). The chicken males were from a nonpurebred Dark Cornish strain selected for a high incidence of parthenogenesis. All birds were housed indoors in individual laying cages and were subjected daily to approximately 14 hours of light and 10 hours of darkness. Each bird had free access to feed and water.

Throughout both experiments all eggs were placed in the incubator within 24 hours of lay. After 9-10 days incubation, the eggs were candled and those without live embryos were opened and the incidence and degree of parthenogenetic developmnt (if any had occurred) were recorded. Eggs with live embryos were returned to the incubator and were candled on each succeeding day until the embryos died. Hybrid embryos reaching 14 or

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more days of incubation could be identified by their dark plumage. The origin, hybrid or parthenogenetic, could not be established for embryos which died before the fourteenth day.

Experiment 1. Five sets of 3 full sisters comprised the group of hens used in this experiment. A Dark Cornish male producing good quality semen was assigned to each trio as its specific semen donor.

The first interval of the experiment comprised a 3-month period during which the incidence and degree of parthenogenetic development were determined in eggs laid by all hens. During the last 4 weeks of this interval, 2 hens of each trio received, twice each week, an intraperitoneal injection of at least 0.1 ml Dark Cornish semen† in 0.2 ml complete Freund's adjuvant. The third hen of each trio was retained as an uninjected control.

The second interval, immediately following the first, comprised a 63-day period during which all hens were artificially inseminated weekly with at least 0.05 ml undiluted semen from the same males which were donors for the semen injections. The first of 8 such inseminations was made 7 days after the last semen injection.

Experiment 2. The next year a similar, but expanded, experiment was conducted to verify and extend the previous results. Twelve sets of 4 full sisters were obtained from the same turkey flock. Twelve Dark Cornish males were obtained again from the flock selected for parthenogenesis. As before, each sister quartet had a male assigned as its semen donor.

This experiment was divided into 3 intervals. The first was a 50-day *preinjection* period beginning at the onset of lay and ending the day of the first semen injection. During this period all eggs were checked for parthenogenesis.

The second interval, immediately following, was a 3-week *injection* period during which 2 hens of each quartet received intraperitoneal injections of at least 0.1 ml whole semen in complete Freund's adjuvant twice each week. Concurrently, a third hen of each quartet received 6 injections of adjuvant alone. The fourth hen was retained as an uninjected control.

The third interval was the *insemination* period which began 7 days after the last injection. Each hen was artificially inseminated once each week for 6 weeks with not less than 0.1 ml whole semen from the previously assigned Dark Cornish males.

After the third interval the semen quality of the males was proven by successful artificial insemination and fertilization of 2 chicken hens each.

Results. Experiment 1. In the 3-month period prior to insemination, the injected hens laid 540 eggs, 76 (14.1%) of which showed parthenogenetic development. Concurrently, the uninjected control hens laid 258 eggs, 44 (17.1%) of which showed similar development. During 33 days immediately following the first insemination, 47 of 92 (51.1%) of the uninjected hens' eggs and 57 of 191 (25.9%) of the injected hens' eggs showed embryonic development. In the final 30 days 14 of 75 (18.7%) of the uninjected hens' eggs and 29 of 157 (18.5%) of the injected hens' eggs showed embryonic development.

Only 3 identifiable hybrid embryos appeared during the experiment. All had Dark Cornish-type plumage and all occurred in the eggs of uninjected hens within 8 days immediately following the first insemination.

Experiment 2. The results of this experiment are summarized in Table I. During the *preinjection* interval, the incidence of parthenogenetic development in eggs laid by hens subsequently to be semen injected, adjuvant injected, or uninjected was 17.0, 17.1, and 20.9% respectively. The incidence of recognizable parthenogenetic embryos in the 3 respective groups was 0.3, 0.9, and 1.7%. None of the 13 embryos encountered survived more than 2 days incubation.

During the *injection* interval, the percent of eggs from all groups undergoing parthenogenetic embryonic development remained low.

The percentage of eggs undergoing embryonic development in the first 21 days of

† In both experiments the minimum semen volumes used for injections and inseminations were dictated solely by the amount of semen produced by the donors.

TABLE I. Incidence of Development in Incubated Eggs from Turkey Hens Before and After Insemination with Chicken Semen. Hen groupings represent injection pretreatment applied 4 weeks before insemination.

Intervals	Hen groups: 24 semen injected				12 adjuvant injected				12 uninjected			
	Eggs No.	Devel. No.	%	Embryos No.	%	Hybrids No.	%	Eggs No.	Devel. No.	%	Embryos No.	%	Hybrids No.	%	Eggs No.	Devel. No.	%	Embryos No.	%	Hybrids No.	%
Preinjection 50 days	807	137	17.0	3	.3	—	—	322	55	17.1	3	.9	—	—	416	87	20.9	7	1.7	—	—
Injection 28 days	268	52	19.4	1	.4	—	—	67	7	10.4	0	0	—	—	197	46	23.4	2	1.0	—	—
Insemination days 1-21	262	87	33.2	11	4.1	3	1.1	60	49	81.7	17	28.3	7	11.7	153	80	52.3	20	13.1	12	7.8
Insemination days 21-42	246	35	14.2	2	.8	0	0	75	21	28.0	1	1.3	0	0	115	25	21.7	2	1.7	0	0

the *insemination* interval rose to 33.2 in semen injected hens, 81.7 in adjuvant injected hens, and 52.3 in uninjected hens. The incidence of recognizable embryos in the three groups was 4.1, 28.3, and 13.1%, respectively. Twenty-two eggs produced hybrid embryos, 3 of 262 (1.1%) from semen injected hens, 7 of 60 (11.7%) from adjuvant injected hens, and 12 of 153 (7.8%) from uninjected hens. No more identifiable hybrid embryos occurred after the eighteenth day. During the next 21 days, the percentage of eggs undergoing development fell to 14.2, 28.0, and 21.7% in the semen injected, adjuvant injected, and uninjected groups. Also, the percent of eggs with recognizable embryos declined to 0.8, 1.3, and 1.7 respectively. No hybrid embryos were encountered.

Discussion. The results of both experiments indicate that intraperitoneal injections of turkey hens with a mixture of chicken semen in Freund's adjuvant prior to insemination by the same semen donors can suppress the ability of their subsequent eggs to undergo hybrid embryonic development. The failure to induce suppression in Exp. 2 by injections of adjuvant alone indicates that semen was the effective component of the injection mixture.

It is clear, however, that the semen injections did not completely inhibit hybridization. Three identifiable hybrid embryos were observed in eggs from semen injected hens in Exp. 2. Furthermore, the total incidence of embryonic development in eggs from these hens for a brief period after insemination exceeded the level of parthenogenetic development immediately before insemination. A determination of whether this partial refractivity resulted from true variability of the hens' responses or from a technical flaw must await the outcome of further research.

The data also show that a long period of weekly inseminations of turkey hens with chicken semen will not necessarily produce equally long-lasting successful hybridization. Rather, they indicate that after an initial period of successful cross-fertilization, there follows a period in which little or no detectable hybrid development occurs in spite of repeated inseminations. In Exp. 1, no identifia-

ble hybrids occurred after the 8th day of the insemination period and in Exp. 2, none occurred after the 18th day. Also, in eggs laid by hens not previously injected with semen, the incidence of development rose to a high level early in the insemination period but later subsided to the level encountered before inseminations began.

There is no way of knowing whether the suppression of embryonic development induced by semen injections and the decline in embryonic development in eggs of both semen injected and control hens after repeated artificial inseminations are the product of similar responses. Moreover, there is no direct evidence on the nature of the mechanism(s) involved. Nevertheless, one hypothesis which should be considered is that in both instances the results may derive from an immunological reaction of the hens to antigens associated with foreign spermatozoa. Certainly, if immunological reactivity were to play any role in infertility, it should do so in a heterologous situation such as this one where strong antigenic differences are expected.

The hypothesis is consistent with earlier findings that in chickens a) transient infertility occurred in hens following a single subcutaneous injection of testicular sperm suspension(4), b) duration of fertility was reduced by repeated intravenous injections of hens with spermatozoa(5) and by repeated artificial inseminations(6), and c) serum antispermatozoa titres increased in hens subjected to repeated artificial inseminations or extended periods of natural mating(6). Similar recent work with turkeys has shown that intramuscular injections of semen and semen nuclear extracts resulted in a drop of 26-53% fertility over a 7-week period. However, no reduction in fertility was detected in uninjected control hens artificially inseminated 5 times each week for 7 weeks(7).

Summary. Eight weekly artificial inseminations of Beltsville Small White turkey hens with Dark Cornish chicken semen were only partially successful in achieving cross-fertilization. Identifiable hybrid embryos were produced only in an 18-day period immediately following the first insemination. Intraperitoneal injections of turkey hens with chicken semen in Freund's adjuvant before artificial insemination by the same semen donors suppressed the ability of their subsequent eggs to undergo hybrid embryonic development. Similar injections of adjuvant alone had no suppressing effect.

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Sensitization Reaction to Carrageenan in the Guinea Pig.*† (31976)

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Carrageenan, a sulfated galactan derived from marine algae, was demonstrated to be a connective tissue growth stimulant in experiments carried out over a decade ago by Robertson and Schwartz(1). Since that time, it has been used extensively as a model of collagen synthesis. The chance observation that a second injection of carrageenan provoked

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