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Use of Male Coturnix Quail in the Laboratory Development of Avian Chemosterilants

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ABSTRACT: Egg fertility of coturnix quail (*Coturnix coturnix*) was measured for 35 to 45 days following single oral doses of six candidate chemosterilants to adult breeding males, adult males whose testes has been regressed by photoperiod manipulation, twelve-day-old male chicks, and six-week-old males with undeveloped testes. Azacosterol, 3-chloro-1,2-propanediol, and mestranol were essentially inactive at 100 or 316 mg/kg in all treatment groups. Breeding adults were sterile for 10 to 25 days after doses of 31.6 mg/kg busulfan, 31.6 mg/kg triethylenemelamine, or 316 mg/kg isopropyl methane sulfonate—all alkylating agents. Adults with regressed testes showed reduced fertility or sterility after treatment with busulfan and triethylenemelamine. These tests indicate that the evaluation of male chemosterilants intended for use on wild avian populations during the period of testicular regression should consist of initial tests on breeding adult quail, followed by tests on adult quail with regressed testes if sterility is noted.

KEY WORDS: vertebrate pest control, azacosterol, bioassay, busulfan, mestranol, propanediols, quail, reproduction, sterilization, sulfonates

Bray et al [*1*][2] demonstrated the feasibility of using the sterile male approach to reduce reproductive success of wild red-winged blackbirds (*Agelaius phoeniceus*). Although their studies were conducted by surgically vasectomizing territorial males, the substitution of chemical agents producing sterility should not invalidate their results. It is, however, impractical to use redwings as the only test animals in the laboratory evaluation of chemosterilants because of difficulty of breeding them in captivity [*2,3*]. Inducing testicular growth by manipulating photoperiods is also of limited

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use because male redwings are refractory or partially refractory to testicular stimulation from July to December [*4*], and their testicular growth in the laboratory is usually less than 50 percent of normal development [*4–6*]. Thus, for routine laboratory studies, it is necessary to use an avian species whose breeding can be manipulated easily throughout the year. This paper describes initial studies with coturnix quail conducted to determine how this species could be used in the routine laboratory phases of developing avian male chemosterilants. Since chemosterilants for redwings most likely would be administered to flocks of nonbreeding males during fall or winter [*7*], the response of male coturnix with undeveloped or regressed testes was of particular interest.

Methods and Materials

Our coturnix were bred randomly from original stock (Random Line 926) obtained from the University of California at Davis. Six quail breeding racks each containing four tiers of six cages were used for all production and fertility studies. Standard procedures [*8*] were followed in the husbandry of the coturnix colony, except that each cage was equipped with a light source providing an average illumination of 107 lx, and chicks were raised in modified three-tier, six-section poultry brooders for six weeks, with either continuous lighting or a photoperiod of 6 hours of light:18 hours of darkness (6L:18D).

Six compounds were selected for study because of known or suspected activity on testicular maintenance or growth: azacosterol [17-β-{(3-dimethyl aminopropyl) methylamino} androst-5-en-3β-ol di-hydrochloride (AZA)], busulfan (1,4-butanediol dimethanesulfonate (BUS)), 3-chloro-1,2-propanediol [α-chlorohydrin (CPD)], isopropyl methane sulfonate (IMS), mestranol [3-methoxy 19-nor-17-α-pregna-1,3,5(10) trien-20-yn-17-ol (MES)], and triethylene melamine [2,4,6-tris (1-azirdinyl)-*s*-triazine (TEM)].

The acute oral toxicity of these compounds was determined on male and female coturnix by the methods described by Schafer et al [*9*].

To evaluate chemosterilant activity, males were gavaged with the compounds in propylene glycol at levels ranging from 5 to 75 percent of the male LD_{50}. One to three levels of each compound and a control treatment of propylene glycol only were administered to test groups of four to ten males. For each compound, at least one group in each of the following four states of testicular development was treated.

Breeding Adult Males

Thirteen groups of six or seven breeding males were dosed and immediately caged individually with fertile females.

Regressed Adult Males

Testes weights in breeding adult male coturnix can be regressed from 3500 to about 10 mg in 28 to 41 days by exposing the birds to a 6L:18D photoperiod. The regressed testes are morphologically similar to those of immature birds that have not bred [*10,11*]. Seven groups of four to seven breeding males were proved fertile under a 16L:8D photoperiod, held under 6L:18D for 45 days, dosed, and immediately paired with fertile females.

Twelve-Day-Old Chicks

Testes in coturnix chicks raised under a continuous 16L:8D photoperiod begin to develop almost immediately after hatching. Through Day 12, however, only resting spermatogonia are present [*12*], and, thus, the testes are morphologically similar to those of adult males in nonbreeding condition. Motile sperm are first produced at an age of 36 to 40 days. Chicks, subjected to a 16L:8D photoperiod were sexed at twelve days of age [*13*], and seven groups of ten males were dosed. At five weeks of age, five to seven randomly selected surviving males in each group were paired with fertile females.

Six-Week-Old Chicks

Testes in coturnix raised under a continuous 6L:18D photoperiod remain undeveloped for at least ten weeks [*14,15*]. When these birds are exposed to a 16L:8D photoperiod, the testes respond quickly and within 24 to 28 days the birds are in breeding condition [*16,17*]. Seven groups of seven males, raised under a 6L:18D photoperiod, were dosed at six weeks of age and immediately paired with fertile females.

All test pairs were maintained under a 16L:8D photoperiod. Eggs were collected from each cage three times each week and incubated for four days in a Jamesway incubator. After incubation, all eggs were opened and recorded as fertile, or infertile if no embryonic development was evident. The effects of treatment were measured by the egg fertility rate (percentage of eggs laid that were fertile) during seven or nine consecutive five-day periods. At the end of the test, the males were killed with carbon dioxide (CO_2), and both testes were removed and weighed as a unit.

Fertility rates for treatments and time periods were tested by a two-way analysis of variance with repeated measures [*18*], and testes weights and time to first fertile egg for treatments by one-way analysis of variance. Duncan's multiple-range test ($P \leqslant 0.05$) was used to separate means for tabular presentation.

Results

Acute Oral LD_{50}

The data on acute oral LD_{50}'s of the six chemicals and the propylene glycol carrier to adult coturnix (Table 1) indicate that TEM was the most toxic chemical to males (LD_{50} = 100 mg/kg), and IMS and MES the least toxic (LD_{50} > 1000 mg/kg). The order and level of toxicity to females was similar to that observed in males. Mortality occurred throughout the seven-day observation period but usually within three days after treatment.

Reproductive Effects

Breeding Adult Males—For all treatments of AZA, CPD, and MES, fertility rates were not significantly different from those of the controls during any of the nine test periods (Table 2). The three remaining compounds, all alkylating agents, induced complete sterility at the highest treatment levels with little or no mortality except for BUS at 100 mg/kg. BUS at 10 mg/kg had no effect on sterility but at 31.6 mg/kg induced temporary sterility for two periods and significantly reduced fertility during four other periods. TEM at 10 mg/kg significantly reduced fertility during three periods, and IMS at 100 mg/kg significantly reduced it during one period.

Regressed Adult Males—Since males with regressed testes were not switched to the 16L:8D photoperiod until they were dosed and paired with females, the fertility rate was zero percent for all treatments during the first two five-day periods. During the remaining five periods, IMS had no significant effect on any parameter measured (Table 3). AZA and MES delayed fertility (probably by delaying testicular maturation or decreasing libido), but, by the end of 30 days, reproductive function in treated birds was similar to that in the controls. CPD-treated males had significantly smaller testes than the controls, but their reproductive function was not impaired. BUS and TEM caused significantly lighter testes and almost complete sterility throughout the test period, but fertility returned to one BUS-treated male during the final period, 31 to 35 days after treatment.

Twelve-Day-Old Chicks—Like regressed adults, these males showed zero percent fertility for at least the first two periods after they were paired with five-week-old females. During the remaining seven periods, AZA, BUS, and TEM had no significant effect on reproductive function (Table 4). Initial fertility in MES-treated males was delayed slightly, but, by the end of the 45-day test period, testes were significantly heavier than in the controls. CPD-treated birds had significantly lighter testes than the controls but no reduction in fertility. Testicular development in IMS-treated

TABLE 1—*The acute oral LD_{50} of six chemosterilants and propylene glycol (PG) to coturnix quail (7-day observation period).*

Chemical	Male		Female		Range of Death Time in h, combined sexes
	No. Birds	Acute Oral LD_{50} and 95 percent Confidence Limits, mg/kg	No. Birds	Acute Oral LD_{50} and 95 percent confidence limits, mg/kg	
PG	2	>2080 (NC[a])	2	>2080 (NC)	...
AZA	8	421 (NC)	8	562 (316 to 1000)	2 to 48
BUS	8	750 (NC)	8	316 (178 to 562)	4 to 48
CPD	8	316 (178 to 562)	8	421 (242 to 750)	14 to 72
IMS	8	>1000 (NC)	8	562 (316 to 1000)	4 to 168
MES	6	>1000 (NC)	6	>1000 (NC)	...
TEM	8	100 (56.2 to 178)	8	133 (75.0 to 237)	14 to 96

[a] NC = not calculable.

TABLE 2—*Reproductive effects when adult breeding male coturnix quail were treated with a single oral dose of a chemosterilant and immediately paired with fertile females.*

Chemical	Dose, mg/kg	No. Pairs Tested	Percent Egg Fertility by Consecutive 5-Day Periods[a]									Percent Male Mortality During Test
			1	2	3	4	5	6	7	8	9	
PG	2080	6	100a	86ab	95ab	80abc	100a	95ab	91ab	95ab	92ab	0
AZA	100	7	97a	100a	86ab	91ab	87ab	91ab	96a	84abc	84ab	14
BUS	100	7	54c	18c	43cd	50bcde	0c	0d	0d	0d	0c	86
BUS	31.6	7	50c	61b	75abc	33de	0c	0d	21cd	53c	73b	14
BUS	10.0	7	93ab	87ab	90ab	100a	91ab	60bc	91ab	94ab	89ab	14
CPD	316	7	100a	76ab	80abc	96a	94ab	100a	50ab	78abc	82ab	43
CPD	100	7	94a	97a	97a	92ab	97a	96ab	100a	91abc	76ab	0
IMS	316	7	81ab	77ab	83ab	39cde	0c	0d	0d	0d	0c	14
IMS	100	7	100a	97a	97a	70abc	55ab	26cd	45bc	93abc	90ab	0
MES	316	7	96a	95a	77abc	94a	96ab	91ab	95ab	74bc	74b	0
MES	100	7	100a	100a	97a	100a	100a	96ab	96a	97a	92ab	0
TEM	31.6	7	54c	6c	18d	18e	5c	0d	0d	0d	0c	0
TEM	10.0	7	50c	16c	59bc	55bcd	43b	59bc	70ab	71bc	71b	0

[a] Numbers followed by different letters are significantly different ($P \leq 0.05$) within each vertical column.

TABLE 3—*Reproductive effects when adult male coturnix quail with regressed testes were treated with a single oral dose of a chemosterilant and immediately paired with fertile females.*

Chemical	Dose, mg/kg	No. Pairs Tested	Percent Egg Fertility by Consecutive 5-Day Periods[a,b]					Percent Male Mortality During Test	Mean Weight of Both Testes, g	Mean No. of Days to First Fertile Egg[a]
			3	4	5	6	7			
PG	2080	7	0a	74a	100a	100a	100a	0	2.876a	18a
AZA	100	7	9a	28b	53b	88a	100a	29	2.532a	20a
BUS	31.6	7	0a	0b	0c	0b	11b	0	1.568bc	...
CPD	100	7	26a	24b	72ab	87a	100a	14	2.080b	18a
IMS	316	7	6a	41b	59ab	85a	86a	14	2.933a	19a
MES	316	4	0a	0b	47b	92a	100a	0	3.071a	23a
TEM	31.6	7	0a	4b	0c	0b	0b	14	0.810c	...

[a] Numbers followed by different letters are significantly different ($P \leq 0.05$) within each vertical column.
[b] Fertility for all treatments during the first two periods was 0 percent.

TABLE 4—*Reproductive effects when male coturnix chicks were treated with a single oral dose of a chemosterilant at 12 days of age and paired with fertile females at 5 weeks of age.*

Chemical	Dose, mg/kg	No. Pairs Tested	Percent Egg Fertility by Consecutive 5-Day Periods[a,b]						Percent Male Mortality During Test	Mean Weight of Both Testes, g	Mean No. of Days to First Fertile Egg[a]
			3	4	5	6	7	8	9			
PG	2080	7	10a	37ab	84a	100a	73a	97a	100a	0	3.061bc	18a
AZA	100	7	0a	36ab	71a	73a	96a	92a	89a	0	3.434ab	19a
BUS	31.6	6	4a	45a	93a	100a	100a	100a	94a	16	2.810cd	18a
CPD	100	7	57a	80a	72a	67a	78a	83a	89a	15	2.386d	18a
IMS	316	7	0a	0b	44a	57ab	50b	50b	50b	29	0.816e	25b
MES	316	5	0a	6b	11b	60ab	100a	100a	100a	0	3.736a	22a
TEM	31.6	7	11a	65a	96a	100a	94a	100a	100a	0	2.722cd	18a

[a] Numbers followed by different letters are significantly different ($P \leq 0.05$) within each vertical column.
[b] Fertility in all treatments was 0 percent during the first two periods.

males lagged behind that in the controls, and fertility was significantly lower during the last three periods.

Six-Week-Old Males with Undeveloped Testes—In these males, the fertility rate was zero percent for the first four five-day periods. AZA and CPD had no effect on reproductive function (Table 5) during the following five periods. Fertility was significantly depressed during one period in MES-treated males, two periods in BUS-treated males, and all five periods in IMS- and TEM-treated males. Testes weights were reduced significantly by BUS, IMS, and TEM.

Discussion

The results allow some tentative conclusions about laboratory procedures for the evaluation of male avian chemosterilants. The four different reproductive stages gave four different test results. Chicks treated at twelve days and paired at five weeks were refractory, showing delayed and reduced fertility only with IMS. Six-week-old males with undeveloped testes were somewhat more sensitive and showed slightly delayed fertility with BUS and MES and reduced fertility throughout the nine-period test with IMS and TEM. We concluded, however, that both these groups were too insensitive to chemosterilants for use in preliminary laboratory evaluations. Adults with regressed testes were somewhat more sensitive, since all treatments tested had significantly lower fertility rates than the controls during at least one period but only TEM produced complete sterility beyond the fourth period. Adult breeding males were the most sensitive to the reproductive effects of the three alkylating agents—BUS, IMS, and TEM. Since our purpose was to find laboratory test procedures that could indicate the effectiveness of compounds on male birds during the fall or winter months when testes are regressed, we preferred to conduct initial tests directly on regressed males. However, economy of time, money, and manpower indicated that it was more practical to concentrate on the adult breeding males, since they take an average of ten weeks from hatch to testable condition versus more than 16 weeks for regressed adults. We, therefore, concluded that initial evaluations should be made on breeding adults followed by tests on regressed adults if sterility was noted.

Of the six chemicals we tested for avian chemosterilant activity, three (TEM, BUS, and IMS), all alkylating agents, produced complete male sterility for varying periods of time. Of the three, TEM showed the greatest activity on both adult breeding males and adult regressed males. TEM has been investigated as an avian male chemosterilant in some limited studies conducted in the United States and Europe. Davis [*19*] indicated that TEM delayed sexual maturation in male starlings (*Sturnus vulgaris*) dosed with 0.2 mg/day for five days during December (when testes were regressed). Although Elder [*20*] and Becker [*21*] indicated that

TABLE 5—*Reproductive effects when 6-week-old male coturnix quail with undeveloped testes were treated with a single oral dose of a chemosterilant and immediately paired with fertile females.*

Chemical	Dose, mg/kg	No. Pairs Tested	Percent Egg Fertility by Consecutive 5-Day Periods[a, b]					Percent Male Mortality During Test	Mean Weight of Both Testes, g	Mean No. of Days to First Fertile Egg[a]
			5	6	7	8	9			
PG	2080	7	16a	55a	86a	83a	93a	0	3.04a	28a
AZA	100	7	25a	63a	71ab	78ab	82a	0	3.007a	28a
BUS	31.6	7	0a	32bc	41bc	70a	80a	0	1.833b	32a
CPD	100	7	7a	45ab	78a	89a	92a	0	3.059a	27a
IMS	316	7	0a	4c	19c	41bc	45b	29	1.565bc	36a
MES	316	7	7a	35bc	84a	94a	100a	0	2.891a	29a
TEM	31.6	7	0a	0c	10c	14c	11b	0	0.781c	30a

[a] Numbers followed by different letters are significantly different ($P \leq 0.05$) within each vertical column.

[b] Fertility was 0 percent for the first four periods.

TEM was not effective in reducing reproduction when fed to pigeons for periods of 10 to 15 days, Lorant and Furnsin [*22*] obtained a patent describing its use as a pigeon chemosterilant. Two field studies with redwings and common grackles (*Quiscalus quiscula*) [*23,24*] indicated a significant reduction in reproduction in marshes when TEM-treated food was continuously available during the breeding season, but neither study differentiated between the effects on the sexes.

Studies with coturnix quail conducted in Massachusetts by D. K. Wetherbee and B. C. Wentworth (unpublished data) indicated that reproductive performance in breeding pairs could be reduced greatly by repeated oral doses of TEM. Further studies indicated that single subacute treatments of males reduced subsequent reproductive performance but incorporation of low levels of TEM in the food of immature and adult coturnix did not. Jones et al [*25*] found that single oral doses of 5 mg/kg TEM and single intraperitoneal doses of 2 mg/kg produced sterility in breeding male coturnix between Days 3 and 10 after treatment. Males treated at 2 mg/kg were also sterile between Days 20 and 38 but then resumed normal reproductive function. Our studies were in general agreement and showed that single oral doses of TEM reduced reproductive performance or induced sterility in adult breeding males, adult regressed males, and twelve-day-old chicks but not in six-week-old males with undeveloped testes.

BUS was investigated in male coturnix by Jones and Jackson [*26*], who used five consecutive daily intraperitoneal (ip) doses of 5 and 10 mg/kg and single ip doses of 20, 30, and 40 mg/kg. Although all treatments produced a biphasic fertility pattern similar to that seen with BUS in mammals [*27*], the 10 mg/kg repeated dose produced complete infertility for only three days. In our studies, a single oral dose of 31.6 mg/kg BUS in breeding birds produced complete infertility during the fifth and sixth five-day periods, but the reproductive function returned to normal levels by the ninth period. The same dose also produced sterility in regressed males and reduced fertility in six-week-old undeveloped males, but it was ineffective in twelve-day-old chicks.

To the best of our knowledge, IMS has not been previously investigated in birds. Our studies indicate that IMS at 316 mg/kg can induce sterility in adult breeding male coturnix and reduce fertility in twelve-day-old chicks and six-week-old males with undeveloped testes, but it does not affect adult males with regressed testes.

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