Avian Contraceptive Tools: One Size Does Not Fit All

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ABSTRACT: Avian contraception is a nonlethal management tool that can be utilized in conjunction with other management techniques to help manage bird populations causing damage. Because management situations vary with respect to the type of damage, species involved, location, and nontarget hazards, it is necessary to develop multiple contraceptive tools to allow management flexibility. DiazaCon™ and nicarbazin have already been developed at the National Wildlife Research Center as avian contraceptives. Although both of these are promising contraceptive agents, more are needed for a wider variety of situations. Current research is focusing on other cholesterol inhibitors, inhibitors of the P450 side chain cleavage enzyme, and aromatase inhibitors. Because cholesterol is needed for steroid reproductive hormone production, inhibition of cholesterol impairs reproduction by preventing the formation of the necessary hormones. The P450 side chain cleavage enzyme is needed to convert cholesterol to pregnenolone, the precursor to progesterone and testosterone. Progesterone is needed for egg formation, ovulation, and oviposition, and testosterone is needed for sperm production. Aromatase is needed to convert testosterone to estradiol, which is needed in females to stimulate the production of egg yolk precursors in the liver. Inhibition of either of these enzymes should result in impaired reproduction. In the future, natural plant products with contraceptive activity, such as neem seed, will also be investigated. Because development of avian contraceptives is time intensive, an efficient screening process needs to be in place. Population models can assist in choosing specific agents to test for a given species and management situation. Although contraception is unlikely to be useful as a stand-alone tool, it can be included in integrated management plans.

KEY WORDS: antifertility agent, aromatase, avian contraception, cholesterol, DiazaCon™, hormones, melatonin, nicarbazin, vertebrate pest control, wildlife management

INTRODUCTION

Because wildlife contraception is increasingly becoming a feasible management tool, it is important to examine the theory behind the science to effectively apply contraceptive tools. It is unlikely that a single contraceptive agent will be effective for all management situations; therefore, it is necessary to develop a suite of contraceptive agents. Development of avian contraceptives is a multi-disciplinary endeavor, including physiologists, wildlife biologists, chemists, and mathematicians.

The type of damage caused by birds can vary, necessitating the choice of very different agents depending on the situation. For example, where damage is related to breeding behavior, such as is the case with brown-headed cowbirds (*Molothrus ater*), the contraceptive needs to affect reproductive behavior. However, if damage is unrelated to breeding behavior, it may be more desirable to affect reproductive potential without affecting behavior. For example, an abundance of urban Canada geese (*Branta canadensis*) cause damage through grazing and large quantities of fecal matter. This damage is unrelated to breeding behavior; therefore, it is unnecessary to affect breeding behavior itself.

The species involved is another important factor in choosing a contraceptive agent. If the species is readily accessible prior to and throughout the breeding season, it is less critical to have a contraceptive that is long lasting. However, if the species involved is only accessible during a narrow window prior to breeding, it is necessary to have a contraceptive that will last the length of the breeding season. Whereas some compounds may be acceptable for use in seasonal breeders, their use in continuous breeders may pose unacceptable health risks. A multi-estrus animal will require a longer-lasting contraceptive agent than a monestrus animal.

The mating system of the species of interest should also be considered. Contraceptives can be designed to affect either one sex or both sexes; therefore, it is important to understand the social structure of the target species. In a polygynous mating system, such as is found in red-winged blackbirds (*Agelauis phoeniceus*), a contraceptive that affects only the female might be preferable. This would allow the male to maintain territories, potentially decreasing the number of extra-pair copulations. Although polyandrous mating systems exist in bird species, they are rare. In a promiscuous mating system, it might be preferable to contracept both sexes. The effect of contraception of one or both sexes on maintenance of the pair bond in monogamous mating systems needs to be evaluated for each species considered.

It is important to know prior to application of a contraceptive what types of nontarget species will be in the area, and when they are likely to breed in relation to implementation of a contraceptive program. If there are many nontargets in the area that cannot be manually excluded from consuming treated bait, an agent that clears quickly from the system is more desirable than a long-lasting agent. However, if there are few nontarget species, a longer-lasting agent could be applied.

Researchers should bear in mind the ultimate goal of registration when choosing and testing contraceptive agents. In particular, health effects on the target animal should be addressed as the agent is being developed. It is necessary to understand the molecular mechanisms of agents being considered to predict what health effects

agents might have, as well as the potential of agents to have secondary hazards. Persistence in the environment should also be considered. In keeping these effects in mind, the researcher should also be developing delivery methods for species of interest to help mitigate potential nontarget and environmental hazards.

PHYSIOLOGICAL TARGETS

Some of the same physiological targets for contraception used for mammals can also be used for birds. Birds offer some additional physiological targets for contraception because they are oviparous rather than viviparous. In addition to reproductive hormones, components of the egg itself related to egg formation and hatchability can also be targeted. These physiological targets may affect either one or both sexes, and this should be kept in mind when developing contraceptives specific to these areas.

Nonsteroidal Hormones

One of the main peptide hormones that has received much attention as a contraceptive target is gonadotropin-releasing hormone (GnRH), a hypothalamic hormone. This is a small protein consisting of 10 amino acids, with only 1 amino acid difference between the avian and mammalian forms. Pulsatile release of GnRH stimulates the release of luteinizing hormone (LH), and to a lesser extent, follicle stimulating hormone (FSH) from the anterior pituitary. Both LH and FSH are necessary for the formation of sperm and oocytes. Inhibition of GnRH inhibits the release of LH and FSH, thereby inhibiting spermatogenesis and oogenesis. Research on inhibiting GnRH has focused primarily on immunocontraception (Levy *et al*. 2004, Miller *et al*. 2004) and GnRH agonists (Baker *et al*. 2004, Herbert *et al*. 2005), although other anti-gonadotropic compounds exist (Gumbinger *et al*. 1981, Kanjanapothi *et al*. 1981).

Another hormone important in controlling the reproductive cycle is melatonin. Melatonin is produced in the pineal gland in response to decreasing amounts of daylight. Melatonin travels to the hypothalamus where it inhibits the production of GnRH. In long-day breeders, such as birds and horses, the lengthening days decrease the amount of melatonin produced, allowing the hypothalamus to produce and release GnRH. During the fall when the amount of daylight decreases, the pineal gland produces more melatonin, which inhibits GnRH, thereby inhibiting reproduction. Compounds that increase the amount of circulating melatonin should therefore inhibit reproduction in long-day breeders.

In short-day breeders, such as deer, melatonin has the opposite effect, stimulating release of LH and FSH. Suppression of melatonin in long-day breeders theoretically should suppress reproduction. The diametrically opposed effects of melatonin on reproduction in short- and long-day breeders may be related to the presence or absence of melatonin receptors in particular areas of the brain (Gerlach and Aurich 2000).

Steroid Hormones

Steroid hormones are produced from cholesterol. The side chain of cholesterol is cleaved by cytochrome P450 enzymes to produce pregnenolone, the precursor hormone to testosterone and progesterone. Testosterone is needed for the production of sperm, and progesterone plays a role in ovulation and oviposition. Testosterone is converted to estradiol, which stimulates the production of egg yolk precursors in the liver. Both progestins (a synthetic compound that mimics progesterone) and antiprogestins have been utilized as methods of inhibiting steroid hormone production, particularly in zoos (Jewgenow *et al*. 2001, Raphael *et al*. 2003). In addition, compounds that inhibit synthesis of steroid hormones by decreasing cholesterol synthesis or by preventing the conversion of cholesterol to pregnenolone have been tested (Yoder *et al*. 2004).

Spermatogenesis

Spermatogenesis can be disrupted by inhibiting testosterone, which is needed for production of sperm in the testes. Various compounds that cause lesions in the seminiferous tubules and epididymus can also affect male fertility by preventing the release of sperm. When choosing to target spermatogenesis, it should be kept in mind that sperm are produced over the course of several months, making it necessary to treat males earlier than females.

Cholesterol Synthesis

As mentioned in the previous paragraph, cholesterol is necessary for the formation of the steroid reproductive hormones. Cholesterol synthesis consists of some 20 steps, several of which have been targeted with cholesterol-lowering drugs for humans (Burnham 2005, Ginsberg 2006). Research in wildlife contraception has primarily focused on downstream targets of synthesis. Desmosterol is converted to cholesterol by the delta-24 reductase enzyme, and this step is one of the primary contraceptive targets for wildlife (Yoder *et al*. 2005*a*).

Egg Components

The egg yolk precursors vitellogenin and very low density lipoprotein (VLDL) are produced in the liver in response to estradiol stimulation. Inhibiting synthesis of these precursors in the liver, particularly VLDL, should result in decreased egg production. In addition, VLDL and vitellogenin can both be altered once they have been released from the liver to prevent their deposition in the developing egg.

The vitelline membrane surrounds the egg yolk, and protects the developing embryo during the initial phases of incubation from the alkaline environment of the albumen. The vitelline membrane can be altered, in particular causing it to become more permeable. This creates an inhospitable environment for the developing embryo, reducing egg hatchability. If the vitelline membrane is altered during its formation and deposition, it may also affect the ability of the egg to be fertilized.

CONTRACEPTIVE AGENTS
Pipecolinomethylhydroxyindane

Pipecolinomethylhydroxyindane (PMHI) affects reproduction by increasing production of melatonin from pinealocytes, and may also affect the testes directly (Fang

and Anderson 1976, Maji *et al*. 1990). The increase in melatonin decreases GnRH production, and mimics what occurs in the fall in long-day breeders. Research shows only 1 - 2 doses of PMHI are needed to suppress reproduction. This compound has been tested in gerbils (*Tatera indica hardwicke*; Chandrakala and Sarkar 1983), musk shrews (*Suncus murinus*; Singh and Dominic 1979) rats (*Bandicota bengalensis* and *Millardia meltada*; Hikim 1985, Dechamma and Sarkar 1987), coyotes (*Canis latrans*; Stellflug *et al*. 1979), spice finches (*Lonchura punctulata*; Maji *et al*. 1990), and agamid lizards (*Calotes versicolor*; Ananthalakshmi and Sarkar 1994). These studies focused on the effects on the male; however, PMHI should also affect females if the mechanism of action increases melatonin production.

Coturnix quail (*Coturnix coturnix japonica*) were used as a laboratory model to investigate the effects of PMHI on both male and female reproduction in birds. Males were treated with either 25 mg PMHI/kg body weight (*n* = 10), 50 mg PMHI/kg body weight (*n* = 9), or 100 mg PMHI/kg body weight (*n* = 8). Females were treated with 50 mg PMHI/kg body weight (*n* = 14) or 100 mg PMHI/kg body weight (*n* = 11). The control group consisted of 13 females and 7 males. Each bird was given a single oral dose of PMHI by gavage (Yoder, unpubl. data).

Male fertility was decreased by 19% at the 50 mg/kg dose and by 21% at the 100 mg/kg dose. However, 5 of the 8 males in the 100 mg/kg group died within 3 hours of dosing, whereas only 1 of 9 males died in the 50 mg/kg group. Females experienced a much higher mortality rate than males. All females in the 100 mg/kg group died within 3 hours of dosing, and half of the females in the 50 mg/kg group died. There was no effect on egg production in the surviving females. Although no toxic effects were reported in the literature for spice finches, these birds were given PMHI subcutaneously, which may have altered its effects. Alternately, Coturnix quail may be more susceptible because they are domesticated and may have an increased rate of absorption in the gut. No adverse effects were reported in mammals.

Aromatase Inhibitors

Aromatase inhibitors are used to treat estradiol-dependent breast cancer (Harper-Wynne *et al*. 2002). Testosterone is converted to estradiol via the aromatase enzyme in the thecal cells of the follicle. Because estradiol is needed for the production of egg yolk precursors in the liver, inhibition of estradiol production should decrease their synthesis in the liver, leading to decreased egg production.

Two aromatase inhibitors (A1 and A2) were tested at a dose level of 50 mg/kg body weight using Coturnix quail. Aromatase inhibitors were administered once daily for 14 days by gavage. Neither aromatase inhibitor affected egg production. Egg production was 0.55 ± 0.1 (mean $\pm$ SEM) eggs/female/day in the control group (*n* = 11), 0.57 ± 0.1 eggs/female/day in the A1 group (*n* = 13), and 0.50 ± 0.1 eggs/female/day in the A2 group (*n* = 12; Yoder, unpubl. data).

DiazaCon™

Originally, DiazaCon™ was investigated in the pharmaceutical industry as a cholesterol-lowering agent for humans (Sachs and Wolfman 1965). Because of some undesirable side effects, the drug was withdrawn from consideration for humans. The poultry industry had an interest in the compound as a means of lowering egg yolk cholesterol, but its utility was limited (Dam *et al*. 1979, Cecil *et al*. 1981). In the 1960s and 1970s, DiazaCon™ was registered with the Environmental Protection Agency as Ornitrol as a reproductive inhibitor for pigeons (*Columba livia*; Schortemeyer and Beckwith 1970, Sturtevant and Wentworth 1970). Research continued during these 2 decades into using DiazaCon™ to inhibit reproduction in red-winged blackbirds (Lacombe *et al*. 1986), grackles (*Quiscalus quiscula*; Fringer and Granett 1970), and house sparrows (*Passer domesticus*; Sanders and Elder 1976, Mitchell *et al*. 1979). Due to re-registration costs, the DiazaCon™ registration was not renewed. In the mid-1990s, the National Wildlife Research Center began testing DiazaCon™ again.

The chemical structure of DiazaCon™ is identical to cholesterol with the exception of 2 nitrogen substitutions for the hydrocarbons at the 20 and 25 positions. DiazaCon™ prevents the conversion of desmosterol to cholesterol, causing an increase in desmosterol and a decrease in cholesterol concentrations in plasma (Dietert and Scallen 1969, Yoder *et al*. 2004). Because many species of interest do not reproduce readily in captivity, plasma desmosterol concentrations can be used as a marker of efficacy (Johnston *et al*. 2003).

Since 1997, DiazaCon™ has been tested on Coturnix quail (Yoder *et al*. 2004), ring-necked doves (*Streptopelia risoria*), brown-headed cowbirds, American crows (*Corvus brachyrhynchos*), mallards (*Anas platyrhynchos*), and monk parakeets (*Myiopsitta monachus*). In all cases, contraceptive effects occurred within 1-3 weeks after the start of treatment (Yoder *et al*. 2005*a*). Daily feeding for 5-14 days inhibits reproduction for approximately 3 months. In addition, DiazaCon™ does not have to be consumed daily to have a contraceptive effect. A bird consuming 5 doses over a 10-day period will have the same level of reproductive inhibition as a bird consuming 5 doses over 5 days (Yoder *et al*. 2005*a*).

AzaCon™

The chemical structure of AzaCon™ is identical to cholesterol except for a single nitrogen substitution for the hydrocarbon at the 22 position. AzaCon™ prevents the cleavage of the side chain of cholesterol, thereby inhibiting production of pregnenolone (Lu *et al*. 1981). Because pregnenolone is the precursor to the reproductive steroid hormones testosterone and progesterone, reproduction is also inhibited.

Coturnix quail were treated with 50 mg/kg body weight AzaCon™ or DiazaCon™. Although AzaCon™ reduced egg production, it was not as effective as DiazaCon™, nor did the effect last as long. Egg production was 0.55 ± 0.1 eggs/female/day in the control group (*n* = 11),

0.42 ± 0.1 eggs/female/day in the AzaCon™ group (*n* = 12), and 0.29 ± 0.1 eggs/female/day in the DiazaCon™ group (*n* = 12; Yoder, unpubl. data).

Nicarbazin

Nicarbazin is an anti-coccidial drug routinely used in the poultry industry since the 1950s to control intestinal coccidiosis caused by *Eimeria* species in broiler chickens. When nicarbazin is accidentally introduced into layer hen rations, egg hatchability and egg production decrease, and the incidence of yolk mottling increases (Polin *et al.* 1957, Jones *et al.* 1990).

Nicarbazin affects hatchability by disrupting the vitelline membrane, causing it to become more permeable (Yoder *et al.* 2006*a,b*). This allows the yolk and albumen to mix, and creates an inhospitable environment for embryonic development. At higher dose levels, nicarbazin can also decrease egg production. It does this by causing degradation of VLDL prior to its being deposited in the yolk, resulting in a lack of follicular development (Yoder *et al.* 2006*a*).

Mallards were used as a laboratory model for Canada geese to test the effects of nicarbazin on waterfowl reproduction. Plasma 4,4′-dinitrocarbanilide (DNC) concentrations ≥3 µg/mL were achieved at doses ≥34 mg/kg body weight (Yoder *et al.* 2006*b,c*). A plasma level ≥3 µg/mL was determined in previous studies as the level needed for contraceptive effects in chickens and mallards (Jones *et al.* 1990, Yoder *et al.* 2005*b*). Hatchability for mallards given 34 mg/kg body weight daily for 14 days was 26 ± 10%, whereas hatchability in the control group was 55 ± 10% (Yoder *et al.* 2006*b*). Hatchability was reduced by 53% in a field study on Canada geese in Colorado (Yoder *et al.*, unpubl. data), and 30% in Oregon at sites where geese consumed the treated bait (Bynum *et al.* 2007). The percent reduction in hatchability was lower in the Oregon study because the geese consuming the treated bait at one study site were not the geese nesting at that site (Bynum, pers. commun.).

Natural Products

Although little research has been conducted using natural products as avian contraceptives, several plant products are known to adversely affect reproduction in mammals (Zeller and Breneman 1981, Adams 1995, Gupta *et al.* 2005). Natural products may be more acceptable to the public as contraceptive agents than synthetic chemicals, and may be associated with fewer environmental hazards.

MODEL SCREENING PROCESS

Based on screening trials at the National Wildlife Research Center, only 25% of the agents screened were sufficiently promising to continue research. Because development of contraceptive agents can be time intensive, an efficient screening process needs to be in place. The initial study should be a dose-response gavage study. This is important for two reasons. First, this allows the researcher to know exactly how much agent each bird is receiving so accurate dose levels can be calculated. Second, if no effect on reproduction is observed, testing more than one dose at a time allows the

researcher to determine whether the agent is ineffective or if the dose was not high enough. To save time and money, the initial test should only examine the effect of the agent on egg fertilization rate, hatchability, and egg production. Because Coturnix quail have a high reproductive output, are not seasonal, and can easily be maintained in the laboratory, they are a good species to use for initial testing.

Once an agent is identified from the initial screening process, the mechanism of action needs to be determined. Only 1 mL of blood can safely be obtained from Coturnix quail at a time; therefore, the use of mallards is desirable for this phase because ≤3 mL of blood can be safely obtained from mallards at a time. Mallards will reproduce in captivity but are seasonal, even if the light cycle is being manipulated. Because of this, the window of opportunity to conduct a breeding study with mallards is limited. However, it is easier to conduct studies to identify molecular mechanisms with mallards due to the greater amount of plasma that can be obtained, allowing the researcher to perform more assays with plasma. Many species of interest do not reproduce readily in captivity; therefore, this phase of testing is also needed to identify biomarkers of efficacy that can be used in any species.

The next phase of testing is a dose-response study with the species of interest. Because species-specific differences in absorption are common, it is necessary to identify a dose level for each intended target species. Once a dose has been identified, a bait acceptable to the target species must be identified. Next, a small-scale field study should be conducted to determine feasibility of using the compound under field conditions for the species of interest, and to determine what nontarget hazards exist. The final step is to conduct a field study to be used as part of the registration requirements for the Environmental Protection Agency. The field studies may require an Experimental Use Permit for the contraceptive agent.

POPULATION MODELLING AS A TOOL

Population models have been constructed to determine the utility of contraception as a management tool. In general, these models have come to similar conclusions. First, it is unlikely that contraception alone can reduce a population as effectively as lethal control (Hone 1992, Barlow *et al.* 1997, Dolbeer 1998). However, it can be used to help maintain a population at a desirable level. Second, contraception will have a larger effect on population reduction for species with a high reproductive potential and mortality rate, than for species with a low reproductive potential and mortality rate (Hone 1992, Barlow *et al.* 1997, Dolbeer 1998). Intuitively, this makes sense from a very basic standpoint. Increasing mortality for a species with a mortality rate of 90% will have little effect on the population, whereas increasing the mortality rate for a species with a 30% mortality rate will have noticeable consequences. The inverse is true for reproductive potential. Decreasing a reproductive rate of 30% will not have as much effect on a population as decreasing a reproductive rate of 90%.

When designing a population model for contraception, several factors should be included. First, compensatory

mechanisms need to be included in the model. Treated animals may exhibit a higher survival rate, and untreated animals may exhibit a higher reproductive potential. Second, the potential effect of contraception on the social structure of the species should be considered, and appropriate variables included in the model. For example, a factor for extra-pair copulations should be included if only one sex is treated, and such copulations are likely to occur. Third, when using models as predictors of management actions, several different models should be constructed. These should include models of the use of lethal control by itself, contraception by itself, and a combination of both. In many cases, the use of both methods may be more effective, reducing the need for lethal control but not eliminating it (Cowan 1996, Bayliss and Choquenot 1999).

Population models can be constructed using information from laboratory efficacy tests and published population parameters. These models allow researchers to evaluate the various levels of contraceptive efficacy obtained with different agents for each species and management situation. Ultimately, this should allow the researcher to make a more informed decision about which contraceptive agents are good candidates for a particular species and management situation, decreasing time spent developing the agent.

MANAGEMENT IMPLICATIONS

Development of avian contraceptives is a time-consuming process; therefore an efficient screening process is needed. Because each management situation is different, it is important to develop several contraceptive agents for use. Avian contraception may never be practical as a stand-alone tool, and in damage management situations, stakeholders are unlikely to accept having to wait several years for a population reduction. However, contraception can be useful as part of an integrated management plan to maintain a population at a given level once it is reduced. Use of contraception in such a plan would likely reduce the need for yearly culling.

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