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Laboratory Evaluation of the Effectiveness of the Fertility Control Bait ContraPest® on Wild-captured Black Rats (*Rattus rattus*)

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Executive Summary

A non-toxic liquid fertility control bait for rats has recently become commercially available (ContraPest® from SenesTech, Inc.). This product contains two chemicals, both of which impair spermatogenesis in male and reduce ovarian ovulations in female rats. We tested the efficacy of this bait in wild-caught adult black rats (*Rattus rattus*) from the island of Hawai'i in a short-term laboratory trial. A control group (n=25) was offered placebo bait and the treatment group (n=25) was offered fertility control bait ad libitum during a 15 day introduction period and during the first of four breeding rounds, for a total of 58 days of exposure. After treatment, all rats were provided placebo bait for the remainder of the study and randomly paired with mates from within their treatment groups for two additional breeding cycles. The treatment group produced no litters during the first and second breeding rounds, while 70% of the control females produced litters. In the third breeding round, 70 days after stopping treatment, the treatment group produced 3 litters (6 pups) compared to 7 litters (24 pups) in the control group. During a fourth breeding round, control rats were crossed with treated rats, producing 6 litters (27 pups) from treated dams and 9 litters (40 pups) from control dams, indicating no apparent infertility effect 99 days after cessation of treatment. This study demonstrates that the reproduction rate of wild-caught black rats can be chemically suppressed if provided ad libitum access to the fertility control bait under laboratory conditions.

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Introduction

Invasive mammals impose tremendous environmental and economic costs through damages to agricultural crops, natural resources, and human health and safety (Bergman et al. 2000, Pimentel et al. 2005). Invasive mammals can be particularly devastating on islands and have led to considerable species decline and extinctions (Courchamp et al. 2003, Doherty et al. 2016), with rats being one of the most damaging taxa of invader (Jones et al. 2008, Varnham 2010, Harper and Bunbury 2015). In addition to the damages they cause as ecological alien species, rats have caused incalculable human and economic costs through damages to agriculture contributing to famine, vectoring zoonotic diseases, and mechanical damages resulting from gnawing and nesting behavior (Singleton et al. 2010, Himsworth et al. 2013). In Hawai'i, invasive black rat (*Rattus rattus*) populations damage crops and food stores, kill native flora and fauna, and are reservoirs and vectors of human disease (Shiels et al. 2014), including leptospirosis and rat lungworm disease (Jarvi et al. 2014, 2015, 2017).

Beyond the pest management results that can be achieved by the components of traditional integrated pest management (IPM) such as sanitation (removal of food sources), habitat management, and physical exclusion, large-scale rat control in protection of agriculture,

human heath, and natural resources has typically involved the use of rodenticides: lethal toxicants formulated into an attractive and palatable bait matrix (Hadler and Buckle 1992; Buckle 1999; Witmer et al. 2007; Witmer and Eisemann, 2007). However, shifting societal values, due to poisoning of nontarget wildlife, animals and children, are increasing the demand for non-toxic, non-lethal alternatives for resolution of human-wildlife conflicts. Traditional anticoagulant rodenticides have aroused concern over poisoning of non-target species, environmental contamination, and humaneness (Eason et al. 2010; Mason and Littin 2003). Wildlife fertility control has been considered as a long-term approach to reduce pest populations and the damages they cause (Miller et al. 1998), and has been predicted to prevent the rapid rebound of rodent populations seen after rodenticide application (Gao and Short 1993).

SenesTech, Inc. (Flagstaff, Arizona) has a U.S. EPA registered, commercial liquid bait formulation, ContraPest®, for fertility control in rats. It contains two active ingredients that target both follicle development and spermatogenesis, blocking reproduction in both sexes. The active ingredient 4-vinylcyclohexene diepoxide (VCD) causes primordial follicle depletion leading to premature ovarian failure (Mayer et al. 2002: Mayer et al. 2004; Mark-Kappeler et al. 2011; Hoyer et al. 2001). Follicular maturation progresses from the primordial stage to primary, secondary, antral and preovulatory in preparation for ovulation (Mayer et al. 2002). VCD targets the finite pool of primordial follicles and once depleted, and the after growing follicles have been eliminated by atresia or ovulation, the ovary fails and is no longer reproductive (Hoyer et al. 2001; Mayer et al. 2002; Mayer et al. 2004; Mauldin 2013). VCD causes primordial follicle loss by interfering with KIT signaling, a key cellular growth and survival pathway within the oocyte (Mark-Kappeler et al. 2011). Atresia is a natural process in the ovary to eliminate follicles not destined for ovulation. VCD greatly accelerates this natural process (Hoyer et al. 2001). The second active ingredient in ContraPestis triptolide, a diterpene triepoxide purified from the traditional Chinese medicinal plant Tripterygium wilfordii. Triptolide specifically stops growing follicles in the ovary and sperm production in the testes (Lue et al. 1998; Hyunh et al. 2000; Xu and Zhao 2010; Xiong et al. 2011; Zeng et al. 2017). ContraPest has very low concentrations of both actives, VCD at 0.09% and triptolide at 0.001%. The combination of these two active ingredients acts synergistically to suppress reproduction in both sexes. Witmer et al. (2017) recently tested the palatability and efficacy of ContraPest fertility control bait in both Sprague-Dawley laboratory rats and in wild-caught Norway rats (*Rattus norvegicus*). Sprague-Dawley rats were provided ad libitum access to the liquid bait, along with ad libitum chow and water for 21 days. Rats that took treatment bait were placed in breeding pairs, as were control rats that took bait without active ingredients. Rats that received treatment bait had no offspring, while 100% of control rats had litters after one breeding round. Similar results of no offspring were found in breeding pairs of wild-caught Norway rats, tested in the laboratory, which took treatment bait and then completed two breeding rounds (Witmer et al. 2017).

A fertility control bait such as ContraPest could be beneficial for controlling the black rat population in Hawai'i. Therefore, the objective of this study was to test the palatability and efficacy of the fertility control bait in wild-caught black rats in a laboratory setting, along with assessing the persistence of the contraceptive effect.

Materials and methods

Animal acquisition, preparation, and disposition

Wild black rats were live-trapped in forested and other conservation areas near Hilo and Volcano, County of Hawaii, under approved state collection permits and landowner permission. Captured rats were transported to the testing facility and dusted with Drione® insecticide (Bayer, Research Triangle Park, North Carolina) to treat for ectoparasites before being housed. Fifty (50) total rats of equal sex ratio were housed individually in numbered metal laboratory cages in a climate and lighting controlled laboratory space at the testing facility (20-22°C, ambient humidity, and 12 hr on/off light cycle). Cages (22 cm x 57 cm x 19 cm) were furnished with PVC refuge tubes sized for one or two rats (isolation or breeding events) and commercially purchased shredded paper bedding with replacement as needed. All rats were fed a maintenance diet of Purina® rodent chow pellets (Nestle Purina PetCare Company, St. Louis, Missouri), and water was provided ad libitum in 250 ml inverted glass bottles with stainless steel sipper tubes throughout the duration of the study. Rats also received wood chew sticks with replacement as necessary.

All rats were individually housed for a minimum quarantine period of 3.5 weeks to ensure that no females were pregnant at the outset of the study phase. Rats were weighed at the beginning of the quarantine period, prior to pairing, and again at the end of the trial phase. All young born during the study were removed upon parturition and euthanized via an overdose of inhalant anesthesia (isoflurane) with subsequent carbon dioxide (CO_2) immersion. Adult rats were euthanized via CO_2 overdose at the end of the study. All animal procedures were conducted within the terms of the study protocol approved by the NWRC

Institutional Animal Care and Use Committee.

Statistical analyses

All statistical analyses and data visualization were performed in the R language for statistical computing (R Core Team 2016). Specific functions and tests are described with methods subsections below.

Bait consumption

Liquid ContraPest, containing the active ingredients VCD and triptolide (hereafter 'active bait'), or an identical formulation lacking the active ingredients ('placebo bait') was offered ad libitum in identical 250 ml inverted glass bottles, as were the bottles providing water. Daily bait consumption was estimated by measuring the bait level within the bottles with a graduated scale. While co-housed for breeding, we continued to record bait consumption though we were unable to determine how much bait was consumed by each paired individual. To test for an effect of the active ingredients on bait consumption (i.e., palatability effects of VCD and triptolide), data from the initial exposure and first breeding cycle phases (the period during which the treatment group received the active bait) were subjected to a linear mixed effects model with bait type (active vs. placebo), 'sex', and study 'phase' (active bait exposure vs. pre- and post-exposure periods) as fixed effects and individual ID as a random effect, '$(1|id)$', to account for multiple repeated measures for each individual. Modeling was conducted using the function '`lmer`' in the package '`lme4`' (Bates et al. 2015), with the model specified as:

$$consumption \sim bait + sex + phase + (1|id)$$

To obtain a p-value for the effect of bait type on consumption, we performed a likelihood ratio test comparing this model to a null model without the bait term in an analysis of variance (ANOVA); the p-value for the χ^2 comparison of the two models is reported as the statistical significance of the 'bait' effect.

Reproductive inhibition trials

Prior to pairing for breeding, all rats were pretreated with the placebo bait formulation for a five-day conditioning period to ensure that rats were familiar with the bait prior to the treatment period. Within each sex group, 13 rats were randomly assigned to the active bait treatment group and 12 were assigned to the placebo bait control group. After the conditioning period, the treatment group was administered the active bait for 15 days while the control group continued to receive the placebo bait. Weight, sex, cage number, and treatment group assignment of each pair was recorded before the initiation of the breeding cycles.

During the first of four breeding rounds (Round 1), the treatment and control groups continued receiving active and placebo bait, respectively. Ten females were randomly paired with ten males within their respective study groups (treated females paired with treated males, control females paired with control males) and the males were placed within the females' cages for mating, with individual IDs recorded for each pairing. The remaining rats in each group continued to be housed individually, to be substituted for rats found to be unfit for breeding due to poor condition, injury, or initial rejection of a male by the female partner during the course of the breeding cycle.

Males were paired with females for 21 to 23 days. If a male was rejected by the female within 24 to 48 h, one of the spare males from the same study group (treatment or control) was substituted for breeding. Females and/or cage papers were examined daily for discarded vaginal plugs as an indication that they had been inseminated. After the pairing period, males were removed and returned to their individual cages. Females were monitored daily for parturition for 23-28 days following removal of males. Within 24 h of birth, pups were removed, counted, and euthanized.

At the completion of the first breeding cycle, the active bait provided to the treatment group was withdrawn and replaced with the placebo bait to determine the persistence of a reproductive inhibition effect. At this time, the treatment group had been continuously exposed to the active bait for 58 days. All rats were provided the placebo bait for the remainder of the study.

For a second and third breeding cycle (Rounds 2 and 3), pairings within study groups were re-randomized without replacement so that males were placed with different females than in previous breeding cycles. For a fourth and final breeding cycle (Round 4), females from the treatment group were crossbred with males from the untreated control group, and treated males were paired with untreated females in order to assess whether treatment of a single sex suppressed reproduction.

After the last round of breeding, all male rats were euthanized and body weights recorded. Testes were excised and weight and length measurements taken. Liver, kidneys (combined), spleen, adrenal glands (combined), and epididymi were excised, cleaned of fats and/or connective tissues, and weighed for future comparative analysis by SenesTech. The testes were individually labeled, placed in mesh biopsy bags, fixed (Davidson's fixative) for 48 hours, and stored in a 10% pH balanced formalin solution in preparation for shipment to the SenesTech lab. Following the last delivery of litters, female rats were euthanized and the body, excised major organs, ovaries and uterus weights recorded.

Statistical differences between counts of litters for treatment and control groups, per breeding round, were tested with Fisher's exact tests. Because litters sizes are count data, they are appropriately analyzed with Poisson or negative binomial generalized linear models. However, data were overdispersed (mean > variance), making them inappropriate for Poisson regression. A negative binomial model would be appropriate; however, exploratory models failed to run, likely due to some test groups having all zeroes. Therefore, simpler basic non-parametric Wilcoxon rank tests sufficed for demonstrating obvious differences, with α set at 0.05 and one-tailed p-values reported under the alternative hypothesis of fewer litters and smaller litter sizes in the treatment group.

Ovarian histology

Ovaries were trimmed of fat and weighed prior to being placed in 10% neutral buffered formalin for tissue fixation, followed by processing. Ovarian tissue was then paraffin-embedded and serially sectioned (5 µm), mounted, and stained with hematoxylin and eosin. Follicles were counted in every 40th section to avoid double counting of small preantral follicles. Follicles were classified as primordial (oocyte possessing a single layer of flattened granulosa cells), primary (oocyte surrounded by single layer of cuboidal granulosa cells), secondary (oocyte encircled by multiple layers of granulosa cells), or antral (oocyte surrounded by multiple layers of granulosa cells as well as containing a fluid filled antral space).

Antral, secondary, and primary follicle counts were determined by the total observed in every 40th section. Conversely, primordial follicle counts were estimated through the formula outlined below, where N_t = total calculated number of follicles, N_0 = number of follicles observed in the ovary, S_t = total number of sections in the ovary, t_s = thickness of the section (5µm), S_0 = total number of sections observed, d_0 = mean diameter of the nucleus of that follicle type (7 µm).

$$N_t = \frac{N_0 * S_t * t_s}{S_0 * d_0}$$

Corpora lutea were estimated by evaluation of every 20th section. Ovarian histology and follicle counts were conducted at SenesTech facilities in Flagstaff, Arizona, under the guidance of B. Pyzyna. A treatment effect was assessed for each follicle type individually using an ANOVA after applying log or square root transformations to improve normality of the data. Normality was assessed by visual examination of data fits to a theoretical normal distribution in quantile-quantile plots (R functions 'qqnorm' and 'qqline'). Due to non-normality of the data, we also explored a treatment effect with the non-parametric unpaired Wilcoxon signed-rank test, which does not assume normality. These traditional tests treat the data as continuous variables (- infinity to + infinity), when they are actually counts (bound at zero) which are more appropriately assessed with Poisson or negative binomial models. Additionally, because all of the objects counted were follicles –assigned to one of five follicle developmental stage – and our overall aim is to assess a treatment effect on follicle counts, we compiled the complete data set with all counts, recording follicle type as a categorical covariate. To account for the likely dependencies among follicle type counts within individuals, we created a random effect variable for rat ID. The Poisson distribution assumes that error means equal their variance; because errors were overdispersed, the negative binomial distribution was used which adds a parameter to account for the excess variance. The resulting negative binomial mixed-effects generalized linear model, using the R package '`glmmADMB`' (Fournier et al. 2012, Skaug et al. 2016), command '`glmmadmb`' with family = '`nbinom`', was specified as:

$$count \sim type + group + (1|id)$$

where '*type*' is a categorical fixed effect for follicle stage, '*group*' is the categorical fixed effect of interest (control versus treated), and '$(1|id)$' is the random effect of rat individual. Reported p-values were based on one-tailed tests with the alternative hypothesis of fewer follicles in the treated group.

Testes mass

Upon necropsy, right and left testes masses were recorded. After summing for total testes mass, values were normalized by final rat body weight into units of milligrams of testes mass per gram of body mass. Normalized testes mass data were approximately normal, and differences in means were tested with an ANOVA.

Results

Bait consumption

Daily bait consumption by sex, study group, and study phase is depicted in Figure 1. There was no effect of inclusion of the active ingredients in the bait formulation on bait consumption (p = 0.739), i.e., no apparent negative effect of active ingredients on palatability.

During this project, males gained significant weight, but there was no discernible difference in weights between test groups of males or females receiving placebo or active-ingredient baits during the treatment phase (Figure 2).

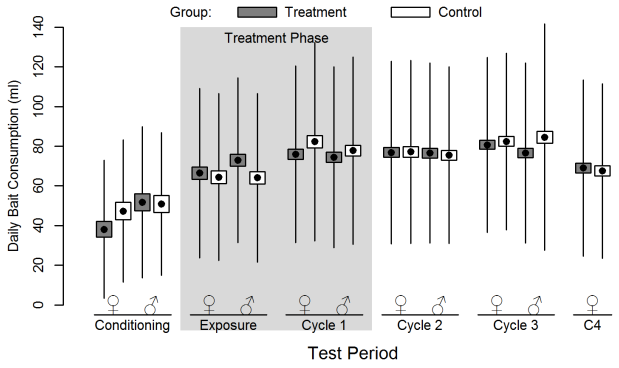

Figure 1: Mean daily bait consumption (points) by sex, study group, and study phase. Boxes around points (means) indicate standard errors of the mean and lines indicate one standard deviation of the consumption values.

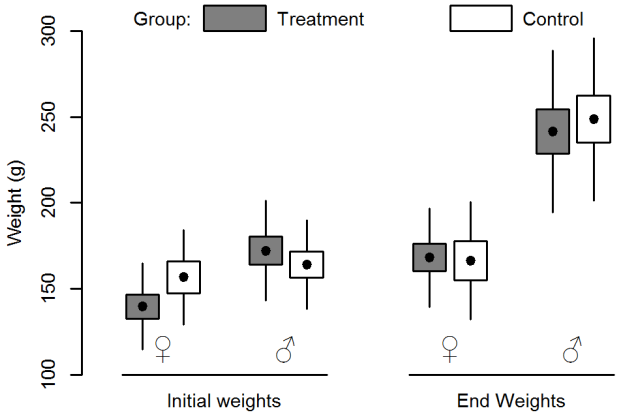

Figure 2: Rat body weights by sex and study group. Dots represent the sample mean, boxes indicate the standard error of the mean, and lines indicate one standard deviation. There were no statistically significant within-sex differences in weight between study groups (α= 0.05).

Reproductive inhibition trials

The first pairing (Round 1) occurred on 15 March 2016 and the last pairing (Round 4) began on 3 August. At the end of the fourth mating period, on 24 August, all male rats were removed from pairs and euthanized. At the end of the last breeding and gestation period, all female rats were euthanized. During pairings, there were only two occasions when males were removed and replaced due to incompatibility/aggression by females. Pairings and litter size details for all four breeding rounds are summarized in Tables 1 to 4.

Table 1: Round 1 pairings and breeding results. Pairings were within study groups (control females x control males, treated females x treated males). Rats were paired from 15 March to 4 April 2016.

Control female	Control male	Pups	Treated female	Treated male	Pups
R74	R76	6	R35	R56	0
R71	R09	0	R58	R30	0
R61	R12	0	R25	R08	0
R18	R26	4	R37	R55	0
R20	R16	3	R38	R57	0
R44	R11	4	R65	R49	0
R42	R05	5	R47	R31	0
R23	R15	0	R64	R53	0
R66	R36	6	R32	R28	0
R43	R04	4	R48	R33	0

Numbers of litters and pups per litter are summarized in Table 5. During the first breeding round, when the treatment group had been exposed to active ContraPest bait for 15 days and was continuing to consume the active bait, there were no litters within the treatment group, while 7 litters, totaling 32 pups, were born to the ten control pairs (70% breeding success). During the second breeding round, which began simultaneous with the replacement of the treatment group's active bait with placebo, there continued to be no reproduction in the treatment group pairs and 70% breeding success (7 litters, 27 pups) in the control group. During both first and second breeding rounds, the difference between 7 control litters and 0 treatment litters was statistically significant (p = 0.002). By the beginning of the Round 3, the active bait had been replaced by placebo for 47 days. During this round, control group reproduction remained at 70%, while treatment group reproduction increased to 30%; though continued reproductive suppression is apparent, the difference between control and treatment group litters was not statistically significant at α = 0.05 (one-tailed p = 0.089). Considering only females that produced litters, the litter sizes in the treatment group (four, one, and one) were smaller than those in the control group ($\overline{X}$= 3.43, SD = 1.18, p $\leq$ 0.05). By the fourth round of breeding, commencing 99 days after removing active bait, treatment group females paired with control group males reproduced at a rate indistinguishable from control group fecundity in previous rounds. There was no apparent suppressive effect when mating control group females with treatment group males, with this group producing the most litters (90% breeding success) and bearing litter sizes indistinguishable from previous control female breeding rounds.

The mean litter size of treated females that did have litters in the first three breeding rounds (ignoring zero values for all females that did not breed) was significantly lower for treated females than control

Table 2: Round 2 pairings and breeding results. Pairings were within study groups (control females x control males, treated females x treated males). Rats were paired from 27 April to 18 May 2016.

Control female	Control male	Pups	Treated female	Treated male	Pups
R23	R09	0	R37	R51	0
R66	R11	3	R38	R28	0
R43	R70	5	R65	R55	0
R74	R14	0	R47	R49	0
R71	R16	0	R64	R56	0
R63	R12	7	R32	R31	0
R18	R36	4	R48	R08	0
R20	R05	1	R03	R53	0
R44	R15	6	R35	R30	0
R42	R26	1	R10	R33	0

Table 3: Round 3 pairings and breeding results. Pairings were within study groups (control females x control males, treated females x treated males). Rats were paired from 13 June to 5 July 2016.

Control female	Control male	Pups	Treated female	Treated male	Pups
R18	R70	3	R64	R31	0
R61	R09	0	R65	R56	0
R44	R05	6	R35	R08	0
R43	R11	2	R37	R53	0
R74	R12	0	R03	R57	4
R42	R14	4	R10	R49	1
R66	R15	0	R48	R51	0
R71	R26	0	R47	R62	1
R23	R16	6	R58	R69	0
R63	R36	3	R25	R33	0

females (p = 0.047; Figure 3a). Litter sizes in the treatment and control groups in the fourth round were not significantly different from controls in the first three rounds. When all females were included in the litter size average, with zeroes included for those without litters (Figure 3b), this pattern remained consistent, though the difference between treatment and control groups in the first three rounds was even more pronounced and statistically significant (P < 0.001).

Ovarian histology

Summary data, along with parametric (ANOVA) and non-parametric (Wilcoxon) test comparisons between control and treatment groups, are reported in Table 6. When each follicle type was considered separately, most ANOVA comparisons between the treatment and control groups were not statistically significant (exception: antral follicles reduced, p = 0.027). Even after transformation, the count data typically did not conform well to the model assumptions of normality. However, in the non-parametric Wilcoxon rank tests most of the comparisons indicated that the follicle counts in the treatment group were significantly lower than in the control group.

The negative binomial mixed-effects model on all follicle counts, which should be considered as a more appropriate model for analyzing count data and considers all follicle count data, indicated lower overall follicle counts in the study group treated with the ContraPest bait formulation (one-tailed p-value = 0.008).

Table 4: Round 4 pairings and breeding results. Pairings were crossed by study group (control females x treated males, treated females x control males). Rats were paired from 3 to 24 August 2016.

Control female	Control male	Pups	Treated female	Treated male	Pups
R18	R31	2	R32	R16	0
R63	R33	1	R10	R05	2
R44	R69	5	R35	R14	0
R43	R51	9	R37	R09	0
R74	R55	1	R03	R15	7
R61	R57	4	R25	R36	6
R42	R53	6	R48	R12	6
R66	R28	0	R47	R70	1
R71	R08	3	R65	R26	0
R23	R56	9	R38	R11	5

Testes mass

There were no significant differences in testes length and width measurements. However, testes mass (normalized to body mass) was significantly lower for treated males (11.3 mg/g) than for those in the control group (14.6 mg/g; one-tailed p = 0.010).

Discussion

These results demonstrate complete reproductive inhibition for wild-caught black rats exposed to ContraPest bait containing the active ingredients VCD and triptolide, ad libitum, under laboratory conditions, for at least 15 consecutive days prior to mating and throughout a 43-day breeding cycle. The inhibitive effect persisted through the second breeding cycle. When paired a third time, 47 days after cessation of treatment, a partial suppressive effect was apparent but not statistically significant, though litter sizes were significantly smaller for the few treatment females that did reproduce. By 99 days post-treatment (Round 4) there was no apparent effect of reproductive inhibition. Given that fertility was rebounding by

Table 5: Litter count and litter size results for female rats (n = 10 per study group). "Bait" indicates whether the treatment group was provided either the active ingredient ContraPest product or the placebo version during the breeding cycle. "Mating" denotes whether females were matched to males by study group (treatment-treatment / control-control) or crossed with males of the opposite study group (treatment-control / control-treatment). "Mean Litter" size is calculated only from females with litters (zeroes not included in the average); however, the Wilcoxon rank test for difference in litter size is based on a litter size of zero for dams without litters.

Breeding Round	Bait	Mating	Litters		Pups		Ctrl	Trt
			Ctrl	Trt	Ctrl	Trt		
1	Active	Matched	7	0**	32	0	4.57 ± 1.05	0**
2	Placebo	Matched	7	0**	27	0	3.86 ± 2.17	0**
3	Placebo	Matched	7	3	24	6	3.43 ± 1.18	2.00 ± 1.41*
4	Placebo	Crossed	9	6	40	27	4.44 ± 2.91	4.50 ± 2.22

One-tailed hypothesis test: * $p \leq 0.05$; ** $p \leq 0.01$

Table 6: Follicle counts, by stage and study group. For ANOVA comparisons, data were transformed to improve normality where necessary. All p-values are from one-tailed tests of the alternative hypothesis of lower follicle counts for the treatment group.

Follicle Type	Control (Min–Med–Max)	Treatment (Min–Med–Max)	p–ANOVA (transformation)	p–Wilcoxon
Primordial (N_t)[a]	122–638–2774	0–83–5338	0.088 (sqrt[b])	0.023*
Primary	0–10–30	0–2–30	0.149 (sqrtb)[c]	0.173
Secondary	0–10–22	0–0–32	0.116 (none)[c]	0.030*
Antral (tertiary)	0–3–8	0–0–10	0.027* (sqrtb)[c]	0.026*
Corpora lutea	3–12–21	0–2–23	0.169 (none)	0.073

[a]Primordial follicle count estimates per formula described in Methods; [b]sqrt = square root transformation; [c]Poor fit despite best transformation, particularly at tails; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

the third breeding cycle, we are unable to draw any useful inference from the cross-breeding of treated and control animals, and the ability to detect any potential sex-specific effect is confounded by the dissipation of the treatment effect.

No evidence of permanent infertility was detected following the 58-day active bait exposure period. However, testes size and follicle counts were lower in treated males and females 120 and 145 days (respectively) after cessation of the treatment. Whether these statistically significant differences translate into any biologically meaningful reproductive effects, or whether more prolonged exposure to ContraPest would lead to permanent sterilization, cannot be inferred from our study. Further studies are needed to assess the effect of long-term exposure on fertility.

Refinements of this or other fertility control baits might afford non-toxic and non-lethal alternatives for protection of agriculture, human health and safety, and natural resources under some management scenarios.

Compliance With Ethical Standards

Conflict of interests statement

This work was conducted at the behest of, and with financial compensation from, SenesTech, Inc., a for-profit corporation with sole financial interest in the ContraPest product, under the terms of a Cooperative Research and Development Agreement (CRADA), APHIS Agreement No. 16-7415-1220-CR. Co-investigator B. Pyzyna is employed by SenesTech. While SenesTech representatives assisted in the establishment of the test protocol and provided materials and consultation during the study, the study director (S. Siers) attests that neither the sponsor nor their representatives influenced the design, conduct, or interpretation of the study in any means so as to compromise the integrity of the study or influence potential financial gain. All activities were conducted at USDA facilities under the supervision of the study director and his representatives, with the exception of ContraPest product preparation and ovarian histology/follicle counts; those data were provided by B. Pyzyna for inclusion in this report, and cannot be vouched for by the corresponding author.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were

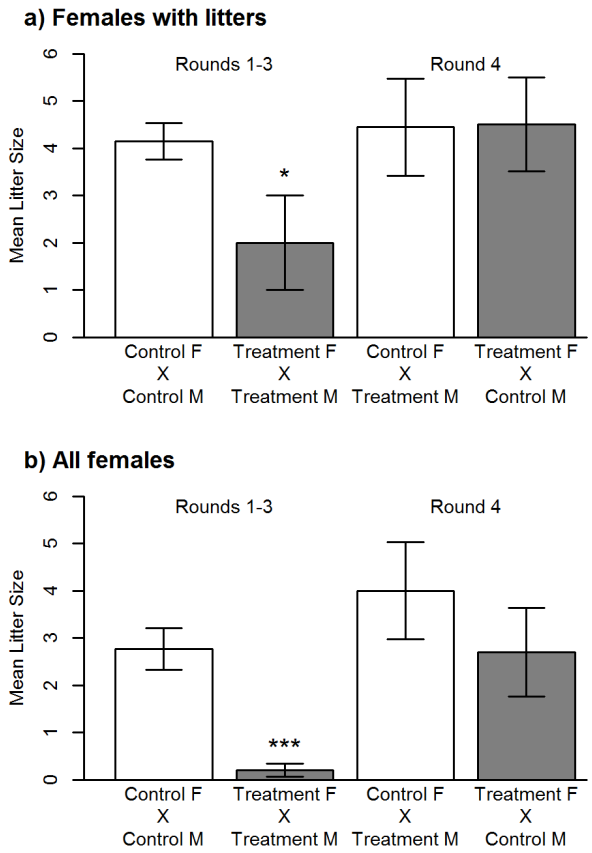

Figure 3: Mean litter sizes for control females (white boxes) and treatment females (gray boxes) ± standard errors of the means. In 'a', litter sizes of zero were excluded from the data, while in 'b' they were included. * $p < 0.05$; *** $p < 0.001$.

conducted.

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