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ORIGINAL ARTICLE

Reproductive responses of rice field rats (*Rattus argentiventer*) following treatment with the contraceptive hormones, quinestrol and levonorgestrol

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Abstract

The rice field rat, *Rattus argentiventer*, is a significant pest of rice in Southeast Asia. Fertility control methods have the potential to provide safe and effective alternatives to control methods that often include indiscriminate use of rodenticides or electric barriers. The aim of this laboratory study was to assess uptake of bait coated with different concentrations of the contraceptive hormones, quinestrol (E) and levonorgestrel (P), delivered alone and in combination (i.e. EP-1) and determine the short-term effects on reproductive parameters of adult male and female *R. argentiventer*. In Experiment 1, 2 concentrations of E, P, and EP-1 (10, 20 ppm) were fed to groups of wild-caught rats for 7 days. In females, both E and EP-1 induced uterine edema. In males, EP-1 reduced epididymis and seminal vesicle weights and lowered sperm motility. However, these responses were inconsistent due to low bait acceptance, especially with increasing concentrations. In Experiment 2, EP-1 (0, 20, 50, 100 ppm) was administered by oral gavage daily for 7 days to male *R. argentiventer*. There were significant reductions in epididymal and seminal vesicle weights for all oral doses of EP-1, in sperm counts for the 50 ppm dose, and in sperm motility for the 20 and 50 ppm doses compared to the control group. To select the optimum dose of EP-1, we must address the poor acceptance of contraceptive-coated baits by rice field rats. Further research is required to improve the palatability of EP-1 and to test its uptake under field conditions.

Key words: ecologically based rodent management, fertility control, food security, pest, rice, Southeast Asia

INTRODUCTION

In Asia, rodent damage to rice (pre- and post-harvest) can cause devastating impacts to smallholder farmers' livelihoods and food security (John 2014; Brown *et al*.

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2020; Singleton *et al*. 2021). Chronic pre-harvest losses of rice to rodents are estimated to be between 5% and 10% per annum, amounting to enough rice to feed 180 million people for 12 months (Singleton 2003). For example, in Indonesia, an estimated 83 462 ha rice (5% of the total harvested rice area) was damaged by rats between 2017–2020 (Indonesian Ministry of Agriculture 2020). In the Philippines, losses of around 10% were estimated to cost rice farmers US$352 per year and represent a substantial amount given the annual average income for smallholder farmers in the region is US$634 per year (or less than US$2 per day; Stuart *et al*. 2011). In Cambodia, a recent study recorded an average 9% rodent damage to rice crops across 4 surveyed provinces (Castilla *et al*. 2020), and in 2 villages, rodent damage led to a 48% reduction in the net income of rice farmers per season (Stuart *et al*. 2020). Another issue of concern is that rice field rodents harbor rodent-borne zoonoses, including hanta virus, rat-borne typhus, plague, and leptospirosis (Meerburg *et al*. 2009; Brown *et al*. 2017a). Leptospirosis, a common zoonosis occurring in humans and animals, is mostly spread through indirect contact with contaminated water; thus, flooded rice fields frequented by rodents pose a significant risk of infection to rice farming communities (Villanueva *et al*. 2014).

The potential for high crop losses and public health risks often necessitates urgent rodent management action and rice farmers in Southeast Asia often apply indiscriminate methods—for example, rodenticides (anticoagulants, zinc phosphide), abamectin-based insecticides mixed with motor oil, and electric fencing—despite their awareness of the hazardous risks to people and non-target animals (Stuart *et al*. 2020). The efficacies of such methods are also questionable, including the common practice of applying zinc phosphide in tropical agroecosystems (Buckle 1999; Hoque & Sanchez 2008). To develop rodent management strategies that are safer and have minimal environmental impact, ecologically based rodent management (EBRM) strategies, such as the trap barrier system, are recommended (Singleton *et al*. 1999, 2004; Brown *et al*. 2006, 2017b; Stuart *et al*. 2020). In Southeast Asia, the rice field rat, *Rattus argentiventer*, is one of the most important rodent pest species of rice in Indonesia, mainland Southeast Asia, and southern Philippines. The breeding season of *R. argentiventer* is closely linked to rice-cropping cycles whereby, during each rice crop, 1 female can give birth to 3 litters, with an average of 10 pups per litter (Lam 1983; Htwe *et al*. 2012). However, if rice crops are planted asynchronously in an area, the breeding season is extended, allowing the pups from the first litter to give birth, which can potentially give rise to 80 rats for every adult female present at the beginning of the season. This number increases exponentially for as long as ripening rice remains available. Thus, EBRM strategies for rice ecosystems in this region generally include approaches to reduce pest population build-up, such as ensuring synchronous planting, community action early in the season, and extended fallow periods (Brown *et al*. 2017b). A key element of the EBRM approach is to limit the availability of food resources and nesting habitat thereby reducing overall numbers and minimizing reproductive potential. Furthermore, the development of effective fertility control agents could represent a major tool for EBRM, particularly in the context of managing rodent population outbreaks (Leung *et al*. 1999; Jacob *et al*. 2008; Massawe *et al*. 2018). Fertility control approaches ideally require that the field application of an agent temporarily or permanently inhibits reproductive activity within a breeding season, primarily targets females, and is humane, environmentally safe, and cost effective (see review, Jacoblinnert *et al*. 2021).

Previous studies (Zhang 2004) have demonstrated that low concentrations of the synthetic steroid hormones quinestrol (E, estrogen) and levonorgestrol (P, progesterone) delivered in combination (i.e. EP-1) can generate long-term (several weeks to months) contraceptive effects in many wild rodent species in China, including Brandt's voles (*Lasiopodomys brandtii*) (Zhao *et al*. 2007), Mongolian gerbils (*Meriones unguiculatus*) (Huo *et al*. 2007), and plateau pikas (*Ochotona curzoniae*) (Liu *et al*. 2012). Recent studies on multi-mammate rats (*Mastomys natalensis*) (Massawe *et al*. 2018) and *Rattus rattus* (Selemani *et al*. 2021) in Tanzania also have indicated the strong potential of these hormones as contraceptives for managing these agricultural pest species. However, these contraceptives have not been previously tested on *R. argentiventer*.

In this study, we conducted a series of laboratory experiments to evaluate the effects of E and P, in combination (EP-1) or alone, on bait consumption and reproductive parameters of male and female *R. argentiventer*. Our aim was to determine whether these compounds have similar inhibitory effects on reproductive function as reported for other rodent pest species and to assess their potential for use in the management of rice field rat populations in rice cropping systems in Southeast Asia.

MATERIALS AND METHODS

Experimental animals

The study was conducted at the Indonesian Center for Rice Research (ICRR) Rodent Laboratory,

Sukamandi, West Java, Indonesia (6°20′S, 107°39′E). Wild live-captured adult male and non-pregnant *R. argentiventer* with head-body lengths between 140 and 200 mm and body weights between 90 and 160 g were used in laboratory experiments (July 2018 to March 2019). These criteria were selected based on the known maturity of *R. argentiventer* (Lam 1983). Following the first 3 batches (out of 5) of males tested, we found that males with testis volumes <11 mm^3/g body weight had low sperm numbers and poor sperm motility in their epididymides. Therefore only males with a testis volume $\geq$ 11 mm^3/g body weight prior to the start of each experiment were used for our analyses and for subsequent tests. Animals ($n = 272$) were trapped using a Linear Trap Barrier System method in Indramayu and Sukamandi in West Java, and from the ICRR research farm. After trapping, animals were weighed, separated by sex and held, sexes separately, in group cages for 7 to 10 days in order to acclimatize them to laboratory conditions. Animals were provided with an unrestricted supply of tap water and unhusked rice. Two weeks before each experiment, animals were weighed, and assessed for body condition. Those deemed to be in good body condition were then transferred to individual cages and fed on standard milled rice grain, with supplements of fresh vegetables, crabs, golden apple snails, and rice tillers.

Bait preparation

For Experiment 1, powdered quinestrol (E) and levonorgestrel (P) (Beijing Zizhutiangong Science and Technology Ltd, China) were weighed to prepare rodent bait at different concentrations (0, 10, and 20 ppm) for each of E, P and EP-1 (1:1). Each weighed hormone was dissolved in absolute ethanol (96%) by heating to 60–70°C. Then the ethanol-contraceptive solution was mixed with a sugar solution made from 5% sucrose in 1000 mL distilled water. The solution was then sprayed onto the plain bait (milled rice) and thoroughly mixed. The control bait was prepared similarly, but without the contraceptives added. All baits were dried under dark conditions at ambient temperature and then stored in dark, dry conditions.

For Experiment 2, stock solutions were first prepared for the 2 active ingredients; Solution A (E), and Solution B (P). One gram of each active ingredient was weighed separately in a glass vial, then 10 mL of 96% ethanol was added to it. Each hormone solution was dissolved by warming to 60–70°C. These stock solutions were stored at 4°C. Each day for 7 days, fresh solutions of EP-1 (1:1; 0, 20, 50, and 100 ppm) were prepared by diluting in corn oil. Rats were lightly anaesthetized with isoflurane and dosed using an 18-gauge gavage needle with a volume (400–850 μL) according to their body weight at the time of dosing.

Experimental design

Experiment 1—Consumption of hormone-coated bait and reproductive responses

A total of 140 males and 45 females were used to assess the uptake of bait coated with different doses (10 and 20 ppm) of E, P, and EP-1 and to determine short-term effects on reproductive parameters. Animals were presented with contraceptive bait for 7 days, where Day 0 is defined as the first day of treatment. During the treatment period, animals were weighed daily, and their general body condition and consumption of contraceptive bait were recorded. In order to prevent the bait falling through to the bottom tray, a rigid plastic sheet was inserted under the wire-based floor of each cage. Each afternoon, each animal was provided with a quantity of fresh bait equivalent to 10% of their body weight. Therefore, if a 100 g animal consumed their 10 g bait, their daily intake would be equivalent to 10 or 20 ppm of their respective hormone treatment. Bait consumption was determined daily. Each morning, any remaining bait was separated from urine and feces, placed in marked trays, dried for 24 h, and then weighed. Control bait samples of each concentration were placed in the animal room overnight, dried for 24 h, and used to correct for moisture loss. The amount of bait eaten each day was calculated by subtracting the amount remaining (after drying for 24 h), from the original amount of bait provided.

On Day 8, females were weighed and then killed by carbon dioxide inhalation for collection and observation of reproductive tracts. The uteri and ovaries were dissected, weighed, and all features such as edema and vascularity of uteri were recorded.

On Day 8, males were provided with a diet of husked rice plus supplements (as described above). On Day 14, 7 days after the end of the dosing period, males were humanely killed, body weight was recorded and their reproductive tracts (testes, seminal vesicles, and epididymides) were dissected and weighed. The length and width of each testis was measured with vernier calipers so that volume could be calculated. One testis and one epididymis were used for assessment of sperm parameters (sperm motility, sperm morphology, and sperm number) according to standard WHO protocols (WHO 2010). The cauda epididymis of each male animal was dissected in a glass petri-dish containing 1 mL of 0.85% normal saline. A drop of the suspension was examined (light microscope,

20× magnification) to determine the proportion of sperm showing normal motility. Another drop of the suspension was taken to prepare a smear for analysis of sperm morphology. Smears were air dried and fixed with a solution of diethyl ether and ethanol (50:50) for 30 min before staining with 10% Giemsa for 30 min, washing with running tap water and drying. Smears were examined under oil immersion at magnification 100×. Two hundred sperm were observed per slide and the proportion showing abnormalities was calculated. For sperm counts, the remaining epididymis samples were placed in glass test tubes and kept at 4°C for 2 h in order to release the sperm. The samples were then diluted 1:10 by adding 9 mL of distilled water and placing in a modified Fuchs Rosenthal (B.S.748) chamber, following the WHO standard protocol to count sperm (WHO 2010).

Additional baseline reproductive data were collected from 20 untreated control males, which were killed and sampled at the end of their acclimatization period. Due to limitations in animal holding facilities, tests were conducted in batches with equal numbers of animals per treatment in each batch. In total, 5 batches of males and 1 batch of females were tested. However, all males (both treated and control) from 2 batches (October and November) were excluded from the analysis due to the majority of control males showing extremely low sperm count and/or motility (results not shown).

Experiment 2—Responses of males to varying concentrations of EP-1 delivered by oral gavage

Bait acceptance by males in Experiment 1 was highly variable as were the reproductive responses observed. To more accurately define reproductive responses to specific doses, we administered EP-1 (0, 20, 50, 100 ppm) by oral gavage to groups of 8 adult males daily for 7 days. Only EP-1 was selected for oral gavage because it gave the strongest response of the 3 hormone combinations assessed in Experiment 1. On Day 14 after the start of treatment, rats were killed and dissected to assess the different reproductive parameters as described for Experiment 1. Animals were provided with husked rice plus supplements (as described above). Females were not assessed in Experiment 2 as we had demonstrated significant reproductive responses to EP-1 in Experiment 1.

Data analysis

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 24 (SPSS Inc., Chicago, IL, USA). Comparisons of mean values between treatments were made using analysis of variance (ANOVA). Pairwise comparisons were conducted using the Bonferroni test. For Experiment 1, separate models were run for the different treatment doses. For the analysis of reproductive parameters, factors included in the model were treatment (E; P; EP-1; control), batch number (all untreated control animals were allocated to a single batch number), and their interaction. Due to the low sample sizes of control males for each treatment dose, the responses of treated animals were compared to the parameters obtained for all control males ($n = 25$).

RESULTS

Experiment 1—consumption of hormone-coated bait and reproductive responses

Bait uptake by male and female R. argentiventer

A total of 56 males and 45 females were assessed for bait uptake (Fig. 1). For both sexes, there was a significant difference in bait uptake between the different hormone treatments for both the 10 ppm (Females: $F_{3,13} = 6.097$, $P = 0.008$; males: $F_{3,25} = 6.459$, $P = 0.002$) and 20 ppm doses (Females: $F_{3,23} = 6.366$, $P = 0.003$; males: $F_{3,22} = 6.539$, $P = 0.002$). For females, animals presented with bait coated with 10 ppm E consumed significantly less bait than the control group ($P < 0.05$), but there was no significant difference in bait take for the other female treatment groups versus the female control group. For males, animals presented with bait coated with 10 ppm E and 20 ppm EP-1 also consumed significantly less bait than those in the control group. Due to this poor acceptance, by males in particular, E and EP-1 bait uptake was not sufficient to achieve the desired treatment dose calculated at 10% body weight.

Female responses to contraceptive hormones

A total of 41 females were used for the analysis of reproductive parameters. This excludes 2 females due to very low bait uptake as well as 2 females that died prior to the end of the observation period. On average, females from all treatment groups lost body mass during the 7-day treatment period. Females treated with 20 ppm EP-1 lost significantly more body mass than the control females ($P < 0.01$; Table 1). At both 10 and 20 ppm treatment doses, E and EP induced increases in uterine weight ($P < 0.05$), with the response being greatest in the EP-1 treated animals. Edema (fluid retention) of the uterus was observed and occurred mostly in EP-1 treated

Table 1 Responses in reproductive parameters of female *Rattus argentiventer* following consumption of baits coated with different doses of E, P, and EP-1 (1:1) in comparison with control females (C) and % change in body mass over the 7-day treatment period

Treatment		*n*	Change in body mass (%)	Ovaries (mg/g body weight)	Uterus (mg/g body weight)	Uterine edema (%)
10 ppm	E	2	−8 ± 2	0.34 ± 0.11†	2.65 ± 0.54*	0
	P	4	−2 ± 10	0.30 ± 0.05	1.54 ± 0.39	0
	EP-1	5	−16 ± 7	0.23 ± 0.03	3.99 ± 0.54**	60
	C	3	−1 ± 14	0.28 ± 0.07	1.28 ± 0.17	0
20 ppm	E	8	−17 ± 6	0.27 ± 0.10	6.12 ± 4.20*	38
	P	8	−6 ± 5	0.27 ± 0.08	3.05 ± 0.71	0
	EP-1	8	−18 ± 6**	0.29 ± 0.11	6.37 ± 2.90**	63
	C	3	−4 ± 2	0.19 ± 0.07	1.66 ± 0.78	0

*$P < 0.05$; **$P < 0.01$ when compared with the control group ($n = 6$) using the Bonferroni test. †Values are Mean ± SD.

Table 2 Summary of male *Rattus argentiventer* reproductive parameters following consumption of baits coated with different doses of E, P, and EP-1 (1:1) in comparison with control males (C) and % change in body mass over the 7-day treatment period

Treatment		*n*	Change in body mass (%)	Testis volume (mm^3/g bw)	Epididymis weight (mg/g bw)	Seminal vesicle weight (mg/g bw)	Sperm count ($\times 10^5$)	Motility (%)	Normal morphology (%)
10 ppm	E	8	−12 ± 6†**	12.4 ± 2.2	8.3 ± 2.2	10.0 ± 4.7**	462.5 ± 363.8	54.1 ± 20.4	68.2 ± 24.7*
	P	10	−4 ± 6	14.6 ± 2.5	8.9 ± 1.2	14.7 ± 5.3	374.0 ± 225.0	65.7 ± 20.9	66.7 ± 21.6*
	EP-1	7	−12 ± 4*	13.0 ± 2.1	6.7 ± 1.4**	6.3 ± 2.5**	207.1 ± 189.8	33.2 ± 27.7*	74.5 ± 19.1
	C	5	−2 ± 4	14.5 ± 2.5	10.3 ± 2.0	18.1 ± 2.5	459.0 ± 198.3	60.0 ± 22.7	87.8 ± 5.9
20 ppm	E	6	−9 ± 8	12.6 ± 2.2	9.1 ± 1.5	10.5 ± 2.8	445.0 ± 75.8*	76.7 ± 7.7	82.5 ± 10.4*
	P	8	−4 ± 3	11.6 ± 1.9	8.7 ± 1.1	13.4 ± 2.8	481.9 ± 97.9	49.1 ± 26.3**	81.6 ± 5.1**
	EP-1	7	−13 ± 5**	10.9 ± 1.4	8.2 ± 1.3	9.5 ± 2.8	410.0 ± 66.0*	45.4 ± 22.3**	86.7 ± 5.7
	C	5	−4 ± 6	11.6 ± 3.2	9.1 ± 1.9	9.2 ± 2.8	602.0 ± 107.4	86.2 ± 8.8	93.7 ± 4.3
Untreated controls		20		14.1 ± 2.1	9.0 ± 1.5	13.8 ± 5.2	501.7 ± 234.7	72.8 ± 26.5	89.5 ± 8.0
All controls		30		13.7 ± 2.5	9.2 ± 1.7	13.8 ± 5.2	511.3 ± 213.1	72.9 ± 24.6	89.9 ± 7.3

*$P < 0.05$; **$P < 0.01$ when compared with the control group ($n = 25$) using the Bonferroni test. †Values are Mean ± SD.

animals. Ovarian weight was not significantly different between groups.

Male responses to contraceptive hormones

Following hormone delivery by bait uptake, a total of 56 males met the minimum thresholds for reproductive parameter analysis (Table 2). This did not include 8 males that died for unknown reasons before the end of the observation period. On average, males from all treatment groups lost body mass during the 7-day treatment period. Males treated with 10 ppm E and 10–20 ppm EP-1 lost significantly more body mass than the control males ($P < 0.05$). When comparing the 10 ppm treatment doses against all the controls males, there was a significant reduction in epididymis weight, seminal vesicle weight and sperm motility following baiting with EP-1 ($P < 0.05$); a significant reduction in seminal vesicle weight ($P < 0.01$) and sperm morphology ($P < 0.05$) following baiting with E; and a significant reduction in sperm morphology following baiting with P ($P < 0.05$) (Table 2).

When comparing the 20 ppm treatment doses against all the control males, there was a significant reduction in sperm motility following baiting with EP-1 ($P < 0.01$),

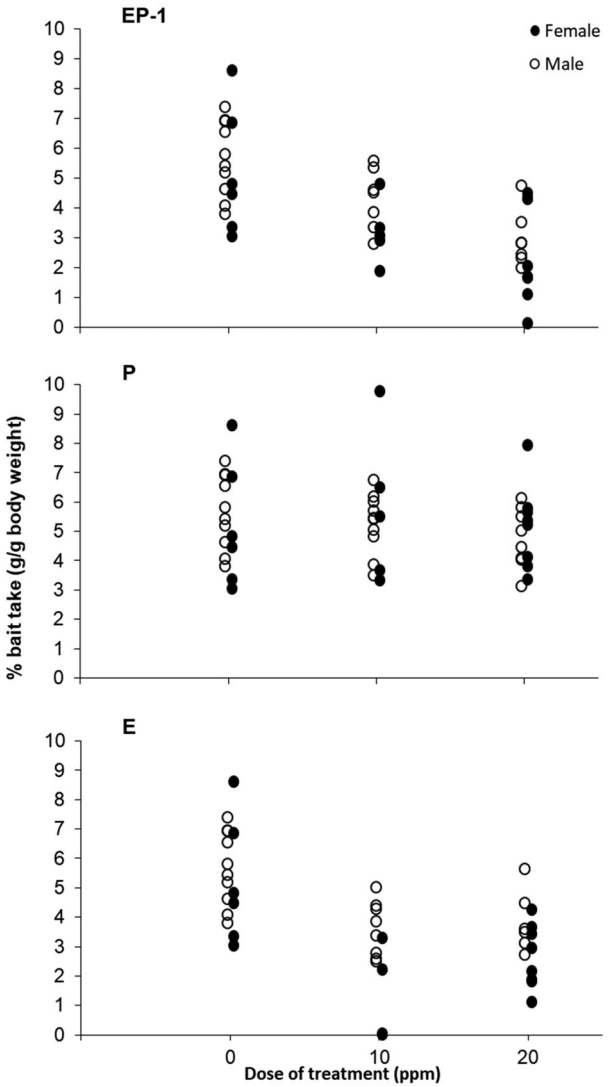

Figure 1 Bait uptake (%; g/g body weight) by male (open circles, $n = 56$) and female (closed circles, $n = 45$) *Rattus argentiventer* for EP-1, P, and E at 10 ppm and 20 ppm treatment doses. Animals in the control groups received control-coated bait (0 ppm).

a significant reduction in normal sperm morphology following baiting with E ($P < 0.05$), and a significant reduction in sperm motility and normal sperm morphology following baiting with P ($P < 0.01$) (Table 2). Given poor bait acceptance, we specifically calculated the total amount of active ingredient consumed by each rat. This did not reveal a clear dose response relationship for any of the reproductive parameters for either treatment (see Figs 2 and 3).

Experiment 2—Responses to hormone delivery by oral gavage

A total of 27 males were used for the analysis of reproductive parameters following oral gavage of EP-1. This excludes 5 treated animals that died during the dosing period. Each of these deceased animals had a white coating on the lungs, pericardium of the heart and in the pleural cavity itself, suggestive of a pre-existing chest infection. On average, males from all treatment groups lost body mass during the 7-day treatment period, with a greater mean loss in body mass for the EP-1 treated groups, but there was no significant difference between treatments. For the 0 ppm dose (i.e. control), a wide range in values were recorded for some parameters (Fig. 4). This was mainly due to 2 outliers for testis weight, epididymis weight and sperm motility, and one outlier for normal sperm morphology.

In comparison with the controls, there was a significant reduction in seminal vesicle weight following all treatment doses of EP-1 by oral gavage ($P < 0.01$; Table 3). There was also a significant reduction in sperm motility following the 20 ppm EP-1 treatment; in total testis volume, epididymis weight, sperm count, and sperm motility following the 50 ppm EP-1 treatment; and in epididymis weight following the 100 ppm EP-1 treatment ($P < 0.05$).

DISCUSSION

Our findings show that when quinestrol (E) and levonorgestrol (P) were delivered in combination (i.e. EP-1) and at sufficient doses, there were clear effects on the reproductive physiology of both male and female rice field rats, *R. argentiventer*. However, when rice field rats were presented with the synthetic hormones on milled rice under laboratory conditions, bait acceptance was highly variable, particularly for E and EP-1 treatments compared to control uptake. Animals receiving only control or P-coated milled rice consumed approximately 3–7% of their body weight per day and generally maintained their body weights, whereas all animals receiving E or EP-1 coated rice consumed less than 6% of their body weight, with an apparent trend of decreased acceptance the higher the treatment dose of EP-1. For some animals, this low acceptance of the hormone-coated bait led to body weight loss (9–13%) and the responses in the reproductive tracts of animals from those test groups were inconsistent. For this reason, we are cautious in our interpretation of the results of this first laboratory assessment of the effects of the synthetic hormones E and P in male and female rice field rats. Variation in bait acceptance by other species,

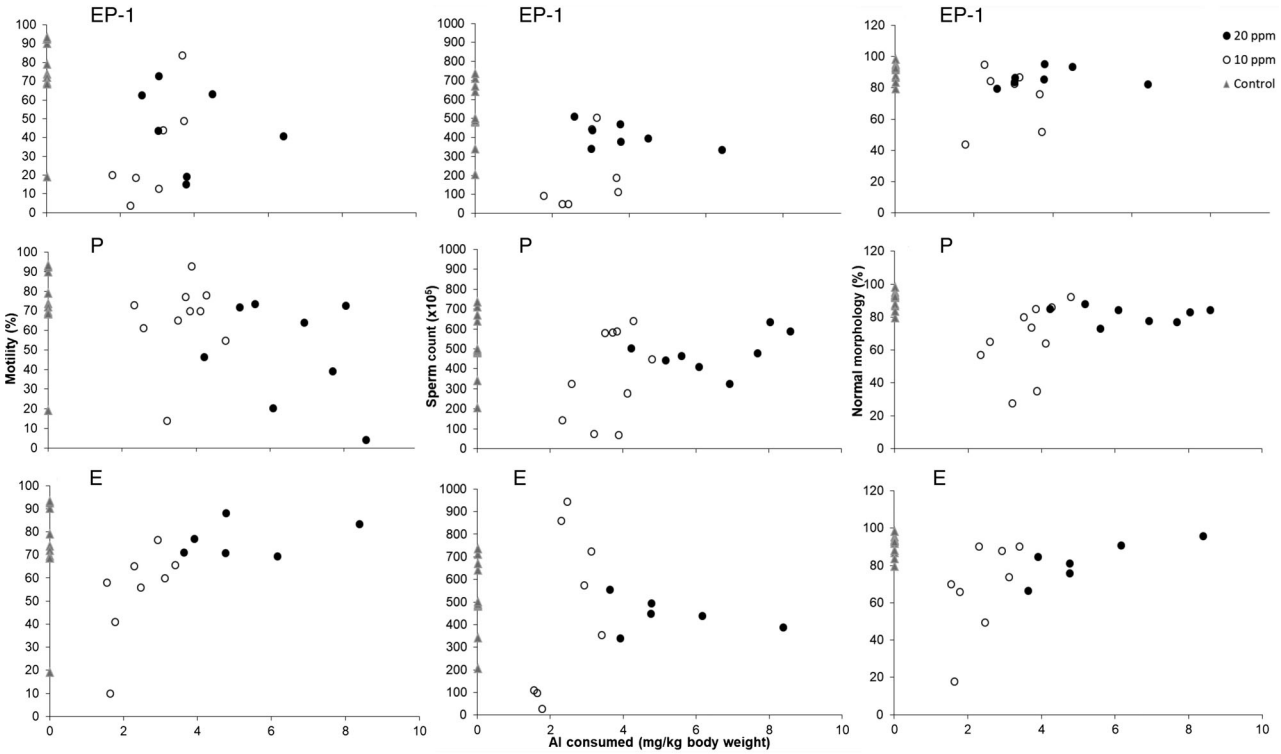

Figure 2 Male *Rattus argentiventer* sperm motility, count, and morphology with respect to actual consumption of hormone (active ingredient, AI; mg/kg body weight).

Table 3 Male reproductive parameters (Mean ± SD) of *Rattus argentiventer* following oral gavage of different doses of EP-1 and % change in body mass over the 7-day treatment period

Dose of EP-1	*n*	Body weight change (%)	Testis volume (mm^3/g body weight)	Epididymis (mg/g body weight)	Seminal vesicle (mg/g body weight)	Sperm count ($\times 10^5$)	Motility (%)	Normal morphology (%)
Control	8	−1 ± 6†	12.3 ± 4.8	7.9 ± 2.0	14.9 ± 3.4	170.6 ± 127.7	57.1 ± 32.3	53.7 ± 25.6
20 ppm	6	−8 ± 7	8.9 ± 3.0	4.9 ± 1.0*	7.6 ± 2.2**	50.8 ± 52.4	5.3 ± 7.4**	37.7 ± 21.1
50 ppm	7	−8 ± 10	5.1 ± 3.0**	4.1 ± 1.3**	7.3 ± 3.5**	20.0 ± 21.0*	4.2 ± 9.3**	24.4 ± 25.9
100 ppm	6	−6 ± 3	7.6 ± 3.7	4.9 ± 1.6*	6.8 ± 2.0**	114.2 ± 153.2	22.4 ± 34.2	47.5 ± 39.3

*$P < 0.05$; **$P < 0.01$ when compared with the control group using the Bonferroni test. †Values are Mean ± SD.

including rodents, has been reported to be problematic with increasing concentrations of estrogenic compounds (Gao & Short 1993; Massawe *et al*. 2018; Selemani *et al*. 2021). Further research is thus needed to improve palatability of baits containing E or EP-1 for *R. argentiventer*. The EP-1 solution in our trials was prepared using a ratio of 1E:1P. Another potential method to reduce adverse effects on acceptance may be to evaluate different ratios of E:P, such as 1:2 (as used for *M. natalensis*; Massawe *et al*. 2018) or 1:3 (as used for *R. rattus*; Selemani *et al*. 2021).

For female *R. argentiventer*, there was an obvious effect of both E and EP-1 in terms of water retention and edema of the uterus at the concentrations tested (10 and 20 ppm), similar to that observed in some other species (Lv & Shi 2011; Massawe *et al*. 2018; Selemani *et al*. 2021), where the extent of edema increased with increasing E or EP-1 concentrations. However, edema is not always observed after treatment (Zhao *et al*. 2007; Liu *et al*. 2012), though this may be related to the tested hormone concentration and treatment duration, as well as the

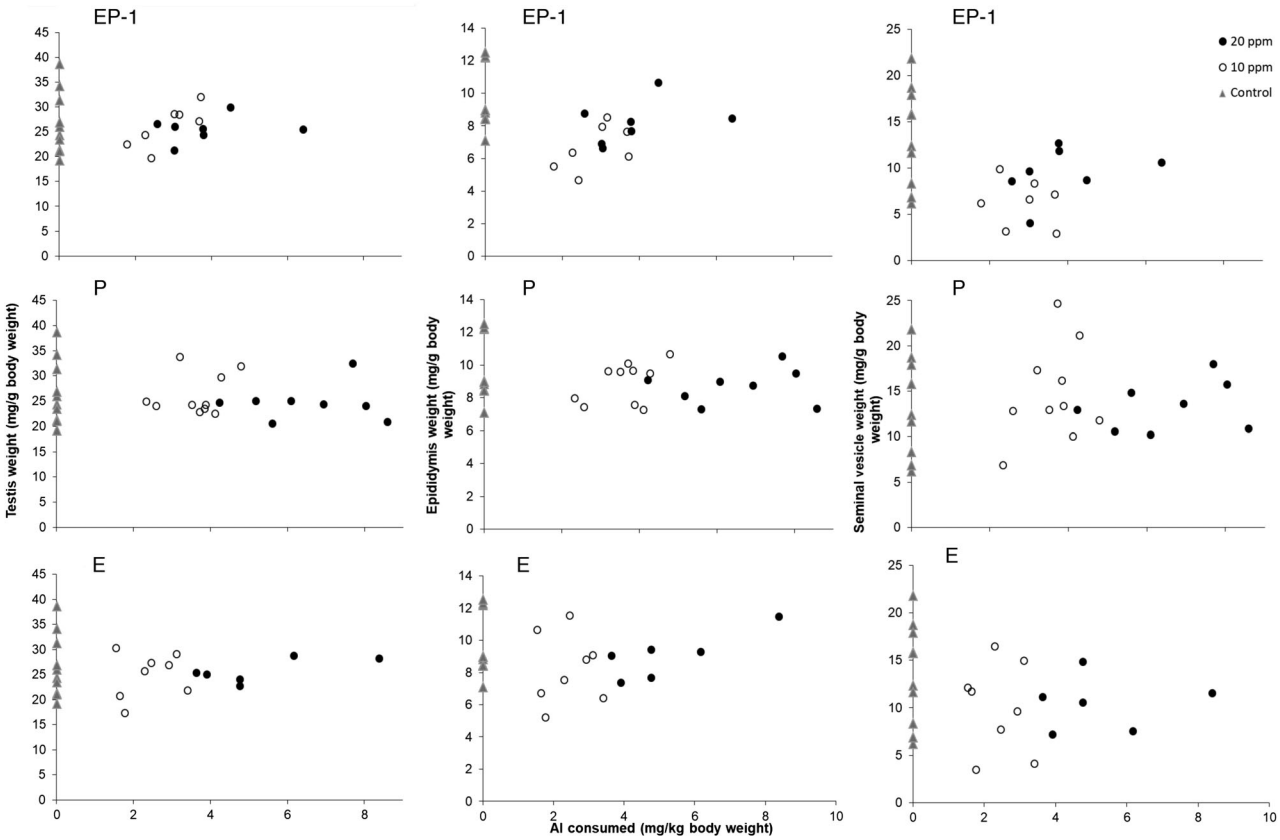

Figure 3 Male *Rattus argentiventer* testis volume, epididymis weight, and seminal vesicle weight in response to actual consumption of hormone (active ingredient, AI; mg/kg body weight).

time of assessment of the reproductive responses after the end of treatment. The extent and duration of edema affects the rate of conception in treated females in Mongolian gerbils after treatment with E (Lv & Shi 2011). Further studies are warranted to investigate the impact of edema on conception rates in female *R. argentiventer* over multiple time-scales.

For male *R. argentiventer*, effects of E and EP-1 following bait consumption were observed in terms of decreases in epididymal and seminal vesicle weights and the inhibition of some sperm parameters. As noted, this response was highly variable in our bait uptake study due to the low level of acceptance of the hormone-coated rice grains. When higher known concentrations of EP-1 were assessed following direct oral gavage, significant reductions in epididymal and seminal vesicle weights were observed 8 days after the end of treatment (by 38–48% and 49–54%, respectively). The strongest effects followed the 50 ppm dose, whereby testis volume, sperm counts, and sperm motility were reduced by 69%, 88%, and 95%, respectively. These results give us confidence that *R. argentiventer* are sensitive to treatment with E, and particularly EP-1. However, contrary to expectation, the responses were weakest following the 100 ppm dose. This was mainly due to 2 outliers, but it is unclear why the higher dose had a lower effect on these 2 individuals. To further complicate the analysis, 2 individual control animals also had particularly low reproductive parameters, highlighting some of the challenges faced when conducting laboratory research with wild *R. argentiventer*.

In comparison with other species, such as plateau pikas (Liu *et al*. 2012), multimammate rats (Massawe *et al*. 2018), and *R. rattus* (Selemani *et al*. 2021), the effects of EP-1 on the reproductive organs of *R. argentiventer* were less pronounced. For example, for *M. natalensis*, EP-1 reduced epididymal and seminal vesicle weights by 60–80%, whereas for *R. argentiventer*, the greatest reductions observed for these parameters were 48% and 54%, respectively. Such differences in reproductive responses between rodent species highlight the need to undertake

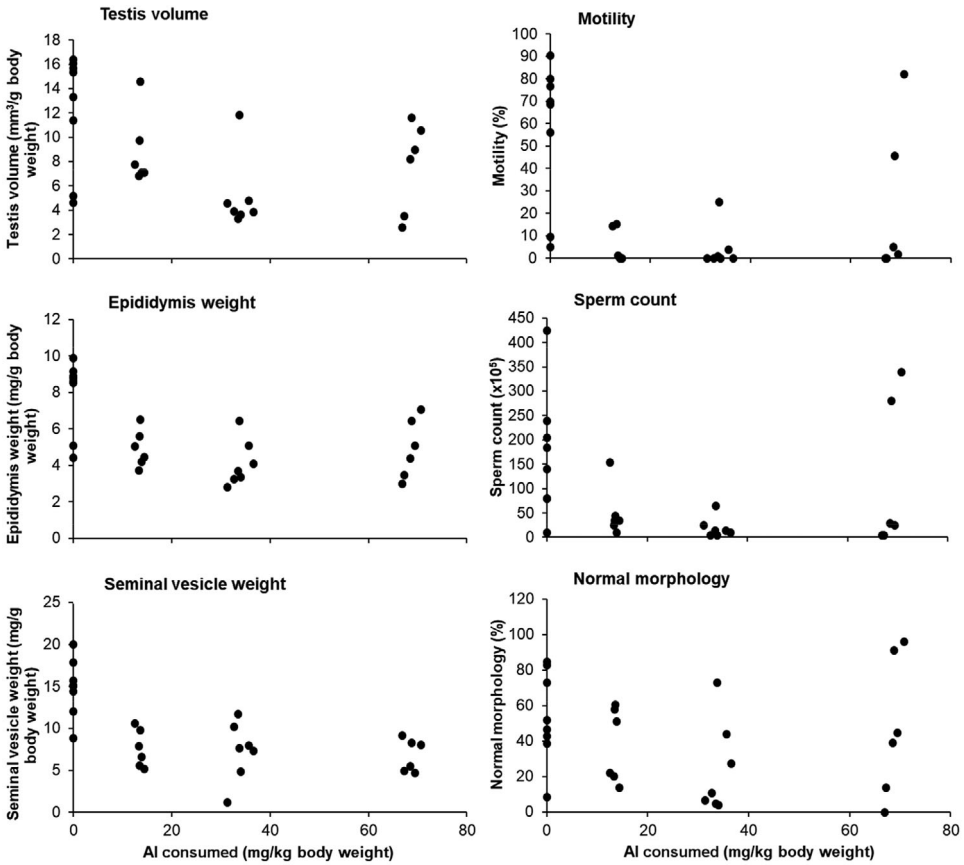

Figure 4 Effects on male *Rattus argentiventer* reproductive organ and sperm parameters in response to oral gavage treatment with different doses of EP-1 (calculated consumption of active ingredient, AI).

controlled trial studies for different target species. However, comparisons of reproductive responses between species can also be difficult as a range of concentrations and combinations of doses have been used. While the combination of EP-1 appears most effective, with the inclusion of P dampening some of the more acute responses to higher doses of E, which ratio of E:P (1:1; 1:2; 1:3) to assess remains problematic. Early studies used E:P at 1:2 (Zhang 2004), but more recent studies have modified the ratio in favor of more P (Selemani *et al*. 2021). It is not clear how these ratios were determined, but a better understanding of how different ratios were selected for other species could help refine the hormone combination (dose and ratio of EP-1) for *R. argentiventer*.

CONCLUSION

For a rodent fertility control method to have field level efficacy on wild rodent populations, there are several important factors that need to be addressed (Chambers *et al*. 1999). These include: (i) the effect on fertility should be sufficient so as to cause temporary or permanent infertility leading to reduced recruitment in the population, (ii) the bait should be attractive to wild rodents in natural settings so that an adequate proportion of the target population consumes the bait, and (iii) the bait should be applied prior to the onset of the breeding season. Our findings show that when EP-1 was delivered at sufficient doses, there were clear effects on the reproductive physiology of both male and female *R. argentiventer*. Overall, the strongest response for all male parameters was produced by the 50 ppm dose of EP-1, but in order to select the optimum dose to use in future studies, we first need to consider the poor acceptance of the bait when mixed with EP-1, especially with increasing concentrations. Based on our findings, a dose within the range of 20 to 50 ppm may provide the optimal dose when balancing efficacy and palatability. However, our results indicate that the acceptance of rice coated in EP-1 at a ratio of 1:1 is likely inadequate for field level efficacy. Thus, the next step is

to identify the optimum bait formulation and E:P ratio for effective delivery in a field situation. Field trials must then be conducted to identify whether uptake of EP-1 bait by wild male *R. argentiventer* is sufficient to induce levels of infertility that lead to reduced recruitment and minimal crop damage. Given that *R. argentiventer* breeding commences at the maximum tillering to booting stage of rice (Lam 1983; Leung *et al*. 1999; Htwe *et al*. 2012), EP-1 baiting would need to be targeted during the early to mid-tillering stages of rice. This would also be a period when food is in short supply, with the dominant food source consumed being monocotyledonous plant parts (Htwe & Singleton 2014). Therefore, the uptake of a bait is potentially favorable at this time. Although the scenario is promising, field trials are urgently needed to test this hypothesis.

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