JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

The use of etonogestrel (Nexplanon®) and aglepristone (Alizin®) for population management of a colony of Rodrigues fruit bats *Pteropus rodricensis*

Gabby J. Drake[1], Veronica B. Cowl[1,2,3], Ian Ashpole[1], Hannah Rowland[1], David White[1], Monica McDonald[4], Julian Chantrey[5], Javier Lopez[1], Yedra Feltrer-Rambaud[2]

[1]*North of England Zoological Society, Caughall road, Upton, Chester, CH2 1LH, U.K.*
[2]*The European Association of Zoos and Aquaria's Reproductive Management Group, Caughall road, Upton, Chester, CH2 1LH, U.K.*
[3]*European Association of Zoos and Aquaria, Amsterdam, Plantage Kerklaan 38-401018 CZ Amsterdam, The Netherlands.*
[4]*AZA Reproductive Management Center at the Saint Louis Zoo, Saint Louis, MO*
[5]*Veterinary Pathology and Preclinical Sciences, University of Liverpool Veterinary School, Leahurst Campus, Neston, CH64 7TE, U.K.*

Correspondence: Gabby Drake, email; g.drake@chesterzoo.org

Keywords: aglepristone; contraception; etonogestrel; megachiroptera; population management; pteropid bats

Article history:
Received: 16 Jun 2021
Accepted: 22 Oct 2021
Published online: 31 Oct 2021

Abstract

Rodrigues fruit bats *Pteropus rodricensis* breed well in captivity and require active population management. Halved etonogestrel implants (34 mg dose) were implanted in 138 female bats either subcutaneously in the brachium, or dorsum, and proved 100% efficacious in both locations. Duration of action was up to 36 months. Implant losses varied by location with 34.8% loss over four years from the brachial site, but only 1.3% loss over two years at the dorsal site. Of bats which lost their implants, 34.5% were found to be pregnant at replacement, indicating reversal is possible. Possible side effects were significant weight gain one year after contraception (mean gain 21.4%) and two possible sequelae to progesterone-based contraception were noted at post-mortem examination of implanted bats that died during the programme; ovarian cysts and mild cystic endometrial hyperplasia. Further data are needed to establish if there is a link to the use of etonogestrel as insufficient pre-contraception data were available. Aglepristone (4.5 mg dose, subcutaneously, twice 24 hours apart) was 75% effective at terminating early pregnancies (<10 mm diameter). Aglepristone failure (confirmed pregnancies that did not terminate with aglepristone) increased as female age increased (odds ratio (OR) 1.7; P=0.02) and there was a tendency for aglepristone failure to decrease with increased pregnancy size (mm), but this was not significant (OR=1.7; P=0.07). No side effects attributable to aglepristone were noted. Breeding was successfully controlled in a population of 239 bats to reduce the colony size over time. At present growth and mortality rates, with 85–90% of females contracepted and a birth rate per sexually mature female of ~0.13/year, compared to an average of 1.08/year prior to the programme, the colony can be managed down to <175 bats over the next 5–6 years. Etonogestrel implants and aglepristone can be successfully used as part of a population management strategy in Rodrigues fruit bats.

Introduction

Rodrigues fruit bats *Pteropus rodricensis* are megachiropteran bats endemic to the island of Rodrigues, near Mauritius, in the Indian Ocean. The species is currently classed as endangered by the IUCN Red List of Threatened Species, with an estimated 20,000 individuals in the wild, but was previously listed as Critically Endangered by the IUCN from 1996–2017. Although the population trend is increasing in the wild, they are vulnerable to climate change and severe weather events, hunting and deforestation (Tatayah 2017). They are a popular and charismatic species kept in captivity for ex-situ conservation breeding and are managed as an EAZA ex-situ programme (EEP) within European zoos.

Pteropid bats generally breed well in captivity, and population size requires active management. Rodrigues fruit bats are believed to be monoestrus with an estimated 4.6–6.3-month gestation and 9-month inter-birth interval (Hayes 1996) and, although seasonal breeders in the wild, they show asynchronous breeding in captivity (Crichton 2000). Chester Zoo has held a colony of Rodrigues fruit bats since 1998 and the colony has steadily grown in size. Regular dispositions to other collections were the main strategy to reduce numbers until recently. Historically, the colony suffered very sporadic deaths due to yersiniosis, caused by the bacteria *Yersinia pseudotuberculosis* and presenting as per-acute deaths with haemorrhagic enterocolitis at post-mortem. From 2010 to 2017, the colony grew from 89 to 236 individuals, at

which point a major outbreak of yersiniosis occurred resulting in 12 confirmed deaths. An additional four deaths were temporally associated with the outbreak but cause of death could not be determined. Since, it was suspected that the larger outbreak was related to sharply increased stocking density, the decision was made to immediately reduce the colony size by culling 27 males. This was followed by a plan to manage the population through the contraception of females, to avoid the negative public perception and lack of EEP support for further culling.

Recent work on bat contraception has focused on surgical techniques (Lafortune 2004; Prud'homme 2020), immunocontraception with Improvest®, a GnRH vaccine, which was effective, but caused severe injection site reactions in male large flying foxes *Pteropus vampyrus* (Mylniczenko 2018), and hormonal methods, including deslorelin acetate, a gonadotrophin releasing hormone (GnRH) agonist, in variable flying foxes *Pteropus hypomelanus* (Metrione 2008) and black flying-foxes *Pteropus alecto* (Melville 2012). While deslorelin acetate was effective in males of both species and in variable flying fox females, the duration of action is only 6–12 months, making them less useful for the long-term management of colonies of this size, due to the frequent need to replace the implants. Since Rodrigues fruit bats are polygamous (Crichton 2000), contraception of males requires all breeding age males in the colony to be contracepted to prevent pregnancies, and vasectomy or castration prevents future breeding. Hayes (1996) found that melengestrol acetate implants successfully suppressed oestrus and prevented pregnancy in female Rodrigues fruit bats over a six-month duration. However, they required surgical implantation, and implant loss was a concern (22% loss rate). Implanted bats also failed to regrow fur at the surgical sites. Melengestrol acetate became commercially unavailable in Europe in 1996. It was superseded by modern synthetic progestogens, such as etonogestrel and altrenogest. In this contraception programme, etonogestrel 68 mg implants (Nexplanon®, MSD Limited) were chosen due to availability, safety, ease of application (non-surgical), and expected long duration of efficacy followed by reversibility. In addition, a progestin form of contraception seemed appropriate, since pregnancy, and therefore progesterone dominance, is the normal hormonal state for a large part of an adult female's life.

Etonogestrel 68 mg implants contain a synthetic progestogen in a radiopaque semi-flexible subdermal implant licensed for use in women. The implants cause slow release of the synthetic progestogen which inhibits ovulation by binding with a high affinity to progesterone receptors. Duration of action in humans is up to three years, after which time the implants should be removed and replaced. It is recommended that a woman is non-pregnant on insertion of the implant, and it does not interfere with lactation (Merck 2020). The majority of the 1392 animals contracepted with etonogestrel implants in the AZA RMC/EAZA RMG Contraception Database were non-human primates (n=1329; 95.5%). It has also been used in chiroptera (n=13, 0.9%), carnivores (n=23, 1.7%), rodents (n=17, 1.2%), marsupials (n=7, 0.5%), and artiodactyls (n=3, 0.2%) (AZA RMC/EAZA RMG Database 2020). Rodrigues fruit bats, along with other pteropodid fruit bats, have high endogenous progesterone levels, even in ovariectomised females and castrated males, suggesting adrenal production is also possible (Crichton 2000). In the AZA RMC/EAZA RMG Database, a dose of 1/3 implant (22.7 mg) has been used in nine *Pteropus* sp. females. Doses of 1/6 and 1/4 of an implant were ineffective in Egyptian fruit bats (Hester van Bolhuis, personal communication), a species approximately half the adult weight of Rodrigues fruit bats. This may be due to insufficient dose, given the high background progesterone levels found in these bat species, therefore, a higher dose of 1/2 an implant (34 mg) was chosen for this programme.

Given the recommendation that females are non-pregnant on insertion of etonogestrel implants (Merck 2020), and that a stark reduction in the numbers of pups born was desired, the decision was taken to chemically terminate early pregnancies in bats prior to contraception. Aglepristone (Alizin®, Virbac ltd) is a competitive progesterone antagonist licensed for termination of pregnancy in the bitch *Canis familiaris* (Virbac Limited 2009), but which has also been successfully used to terminate pregnancy in domestic cats *Felis catus*, rats *Rattus norvegicus domestica*, rabbits *Oryctolagus cuniculus*, goats *Capra aegagrus hircus*, cattle *Bos taurus* (Gogny 2016), sheep *Ovis aries* (Ozalp 2016), African wild dogs *Lycaon pictus*, and lions *Panthera leo* (Bertschinger 2016). The dose used on the bats was extrapolated from these studies.

The aim of this contraception programme was to effectively curb the growth of the colony of Rodrigues fruit bats and reduce the risk of further disease outbreaks due to high population density. The population management strategy aimed to maintain bat behaviour, slow population growth and retain the existing age structure of the colony with some replacement of animals and rotation of breeding. Ultimately, the plan was to gradually reduce the colony size over a 5–10-year period to around 175 bats while maintaining good health and genetic diversity.

This paper specifically (1) assesses the efficacy and appropriateness of a 1/2 etonogestrel implant for contraception (comparing implant loss rates from two different placement sites) and explores the likelihood of future reversibility from these implants, (2) investigates whether any short-term or long-term side effects may have resulted from use of either etonogestrel or aglepristone, and (3) assesses the efficacy of and health risks associated with the use of aglepristone for pregnancy termination.

Materials and methods

Housing

The colony of Rodrigues fruit bats are housed in a public walk-through free flight indoor exhibit approximately 20×30×9 m or 5,400 m^3 in volume. The Rodrigues fruit bats share this exhibit with a colony of 150 to 450 Seba's short-tailed fruit bats *Carollia perspicillata*.

Data collection

Bats were censused approximately every six months during the main reported period from 2017–2020. At a census, all Rodrigues fruit bats were caught, either by trapping at feed stations, or by netting, and microchips were checked to identify individual animals. Any unidentified juveniles or infants found, including un-weaned pups on dams, were implanted with a microchip. A proportion of bats were weighed at each census. Census data was stored in the Zoological Information Management System (ZIMS) (Species360, Bloomington, MN, 55425 USA). All bats that died during the reported period were submitted for necropsy. Necropsy and medical procedure data were recorded in ZIMS medical.

Contraception

Preliminary examinations of sexually mature female bats (>1 year old) in March 2017 demonstrated that pregnancy was only detectable by ultrasound from approximately 3 mm fluid filled internal dimension of the uterus and that bats could take up to 6 weeks after separation from males for pregnancies to be detectable. The contraception programme occurred in three phases detailed in Figure 1.

All sexually mature female bats were manually restrained and assessed for pregnancy at every census. Pregnancy detection was by palpation of the abdomen and/or ultrasonography (16 MHz L8-18i 'hockey-stick' probe, LOGIQ e, GE Medical Systems Co. Ltd, UK) using both transverse and longitudinal sweeps of the abdomen. Bats were classified as no detectable pregnancy (NDP), early

Journal of Zoo and Aquarium Research 9(4) 2021
https://doi.org/10.19227/jzar.v9i4.627

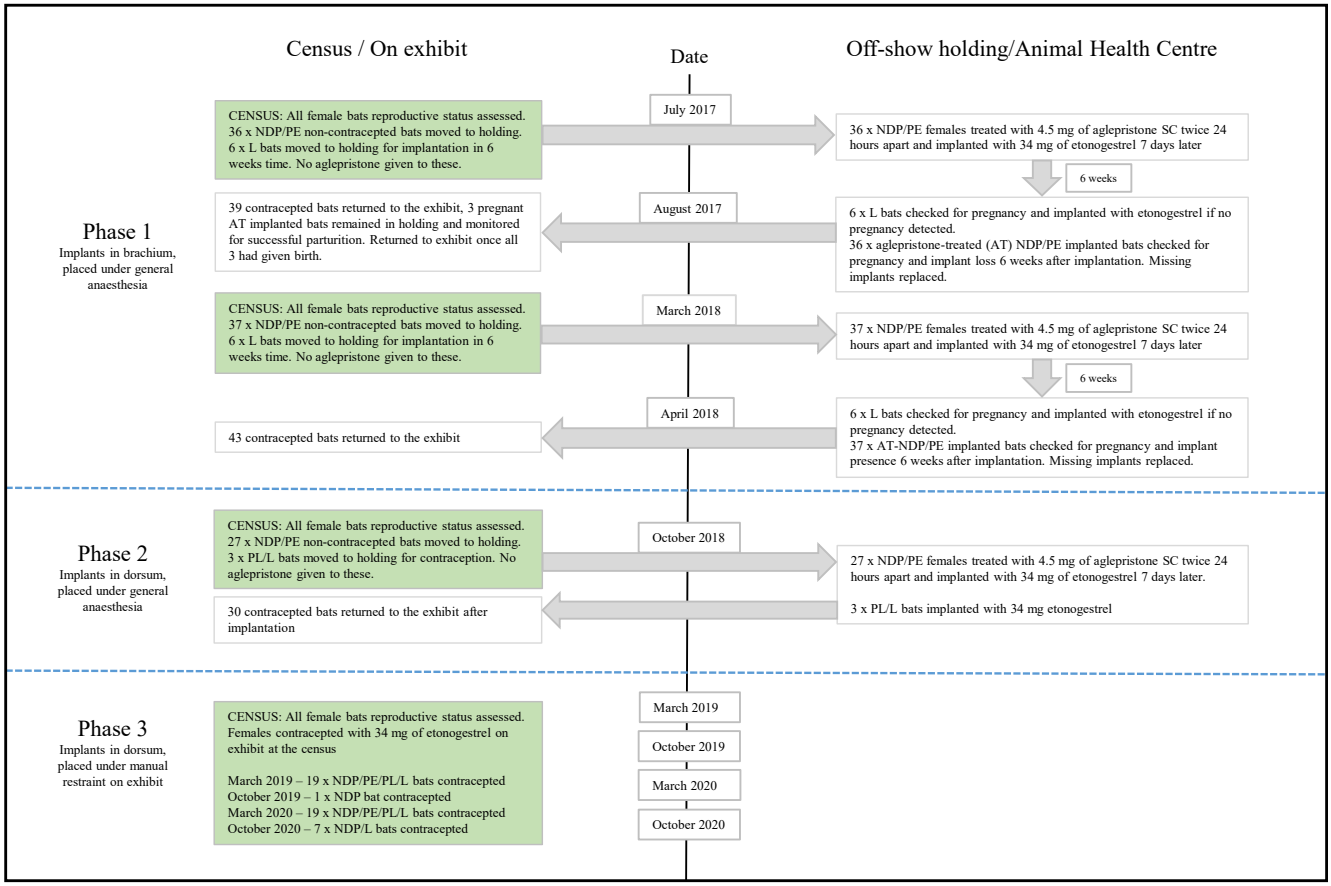

Figure 1. Schematic of methods used in the contraception programme for Rodrigues fruit bats showing the timeline of actions and drugs used for females of different pregnancy statuses through the three methodology phases. NDP no detectable pregnancy; PE early pregnant; L lactating; SC subcutaneously; PL late pregnancy; AT aglepristone treated.

pregnancy (PE), defined as a <10 mm fluid swelling in one uterine horn +/− foetus, late pregnancy (PL), defined as >10 mm uterine fluid swelling with foetus, or lactating (L), defined as females carrying an un-weaned pup. Some lactating females were not scanned to minimise disturbance to the pup, but were presumed non-pregnant.

In Phases 1 and 2, NDP and PE bats were treated with aglepristone prior to contraception (4.5 mg, twice, 24 hours apart, Alizin®, Virbac ltd), and given 7 days minimum from first injection to clear uterine contents. Late pregnant and lactating females were not treated with aglepristone as it was decided not to terminate pregnancies that would be unlikely to be resorbed, and because aglepristone can interfere with prolactin production and lactation.

In Phase 1, only bats believed to be non-pregnant were contracepted and bats received a six week follow up pregnancy check, but in Phases 2 and 3 bats of any reproductive status were contracepted and 6-week follow-up pregnancy checks were stopped.

For contraception, etonogestrel 68 mg implants (Nexplanon®, Merck Sharp & Dohme Limited, UK) were sectioned in half under sterile surgical conditions and inserted into a standard 2 mm sterile microchip needle after removal of the microchip (Peddymark 2×12mm ISO-transponder, PeddyMark Ltd, UK) and implanted subcutaneously in either the medial right brachium or dorsum. All contracepted bats were checked by palpation for presence of the implant at every census. Implants not palpable were confirmed missing by radiography and replaced. In Phases 1 and 2, bats missing implants were also treated with aglepristone prior to replacement, to terminate any early pregnancy.

Additional bats were contracepted at each census, gradually increasing the percentage of the population on contraception. Data up to October 2020 are included in this paper.

Statistics

Census data were extracted from ZIMS and compiled in Excel® (Microsoft Corporation, Redmond, Washington, USA). Contraception data were maintained in Excel®. Statistical and graphical analysis was done using Excel® and R (R Core Team, 2013).

Aglepristone treatment success was analysed, using data from bats with confirmed pregnancies only, by binomial logistic regression with pregnancy termination as the dependent variable and age and weight of mother (equivalent to dose) and size (mm) of conceptus at administration of aglepristone as the explanatory variables. P was set at <0.05.

Descriptive statistics were used to examine the etonogestrel contraception and pathology data. Correlations between age and bat weight were analysed using Spearman's correlation for non-parametric data. Bat weights were compared pre- and at least

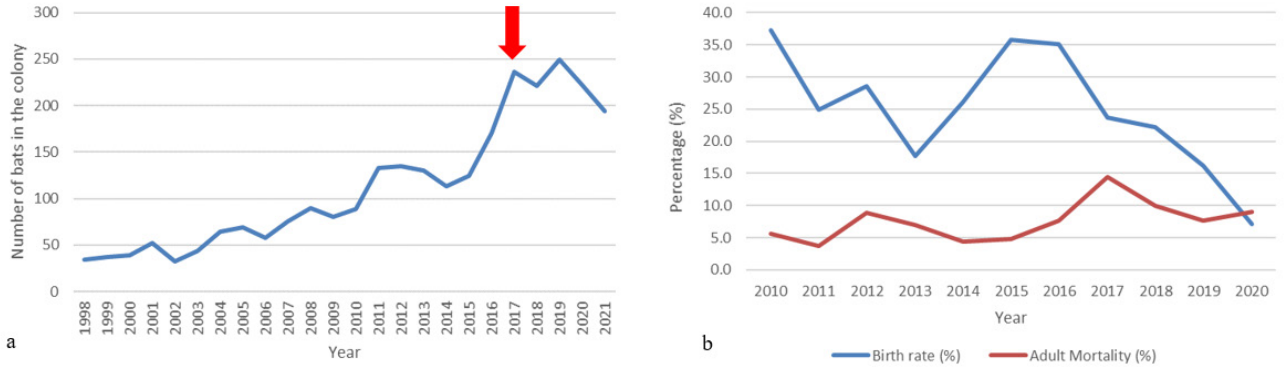

Figure 2. Population changes over time in the Rodrigues fruit bat colony at Chester Zoo. Figure 2a. annual census of Rodrigues fruit bats since creation of the colony in 1998. Red arrow indicates the start of the contraception programme in 2017. Figure 2b. Birth rate (%) and natural (non-cull) adult mortality rate (%) for the Rodrigues fruit bat colony between 2010 and 2020.

one-year post-contraception. Weights of 1-year-old bats were excluded from the pre-contraception data, as these younger and smaller bats skewed the data. Weight data were assessed and found to be normally distributed, and with comparable variances, so were compared using a two sample T-test. P was set at <0.05.

Results

Population changes over time

The contraceptive protocols used in this programme successfully decreased birth rates within the population (Table 1; Figure 2a). Despite a slight increase in colony size in 2019, death rates exceeded birth rates for the first time in 2020, 3 years after the initiation of the programme (Figure 2b). By the end of 2020, 88.3% of sexually mature females were contracepted.

Etonogestrel

Efficacy

A total of 163 bouts of contraception have been initiated in 138 female Rodrigues fruit bats since March 2017 (Table 2). A total of 107 bouts lasted for at least 12 months, the remaining 56 bouts either had implants lost before 1 year or had been in situ for less than 12 months at the time of data analysis. No implants were removed during the reported time period. Bout duration varied as bats were sequentially recruited in to the contracepted population at each census and were not all implanted at the same time. Of the 107 bouts lasting at least 12 months (i.e., with two follow up censuses) no bats were found to be pregnant at 12 months after receiving an implant or at any subsequent census where implants were still in place, giving up to 36 months duration of contraception in bats implanted in March 2017 (Figure 3).

Table 1. Demographic data relating to female Rodrigues fruit bats between 2010 and 2020 including population census, births for that year, births per sexually mature female for that year and percentage of sexually mature females contracepted on the 1st January of each year.

Year	Census (Total, [male.female. unknown])	Births	Births per sexually mature female	Sexually mature females contracepted on 1/1/ year (%)
2010	89 [37.52]	53	1.18	0.0
2011	133 [58.75]	44	0.94	0.0
2012	135 [57.78]	54	0.96	0.0
2013	130 [66.64]	28	0.72	0.0
2014	113 [46.67]	40	0.73	0.0
2015	124 [58.66]	69	1.57	0.0
2016	170 [77.93]	92	1.48	0.0
2017	236 [110.126]	73	0.86	0.0
2018	221 [75.146]	63	0.57	39.1
2019	249 [86.163]	48	0.36	69.4
2020	222 [72.150]	17	0.13	75.4
2021	194 [69.124.1]	-	-	88.3

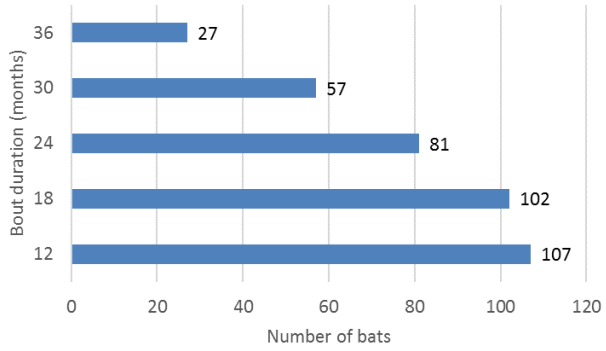

Figure 3. Number of Rodrigues fruit bats with different contraception bout durations at the end of 2020. All bouts had 100% efficacy.

Dose

The etonogestrel dose given was half an implant (34 mg) for all bats, but due to substantial variation in body weights doses ranged from 79.3 to 161.9 mg/kg with a mean dose of 112.9 mg/kg and standard deviation of 14.7.

Comparison of implant sites

Of the 163 bouts of contraception, 87 implants (53.4%) were placed subcutaneously medially in the right brachium and 76 (46.6%) were placed subcutaneously over the dorsum. Thirty-one of the 89 implants placed in the brachial region were subsequently confirmed to have been lost (34.8%; Table 2). Two implants were lost without access to males, so are counted as one contraception bout, but two implant losses. One of the 76 implants placed in the dorsum was subsequently confirmed lost (1.3%) within 6 months of insertion. There has been up to 24 months of follow-up for bats implanted over the dorsum. Implant loss from the brachial region was identified anywhere from 1–33 months post implantation, with implants located at earlier censuses prior to detection of the loss (Figure 4a). Median timeframe to loss was 6–12 months and 94% of implants were lost within 18 months of insertion.

Reversal after implant loss

Twenty-nine implant losses occurred while females had access to male bats. Of these 29 bats, 10 had confirmed early or late pregnancies at the time implant loss was detected, between 0 and a minimum of 15 months post insertion, a reversal rate of 34.5% (Figure 4b). Another bat had a small pup, but the implant was lost within 6 months of insertion, so it is suspected the bat went undetected as pregnant at the time of insertion.

Possible side effects

Thirty-nine bats were pregnant while implanted with no health issues noted for the females. Twenty-three of these were implanted while pregnant (PE=7, PL=16), 16 were aglepristone failures. Twelve of these bats were subsequently found lactating with pups, suggesting successful birth and early rearing. The remaining 27 bats that were pregnant when implanted were not found subsequently with pups. It is unknown whether they aborted or gave birth and failed to rear the pup or the pup reached independence before the next census. One female pup born to one of the three original aglepristone failure etonogestrel-implanted females showed a degree of genital masculinisation upon maturity.

The weight of bats 1-year post-contraception (mean=374.4 g, range=207.0–499.0 g) was significantly greater than non-contracepted bats (mean=318.1 g, range=210.0–429.0 g; t=8.34, P<0.0001 (2-tailed), df=178.69) aged two years and over. The mean weight gain after contraception was 68.1 g (+21.4% body mass), where two weights were available for the same bat.

Between January 2009 and December 2020, 217 Rodrigues fruit bats died or were euthanised. The main identified causes of death are detailed in the Supplemental Figure 1. No deaths were attributed to reproductive pathology in this time period and no reproductive abnormalities were noted prior to 2017, although

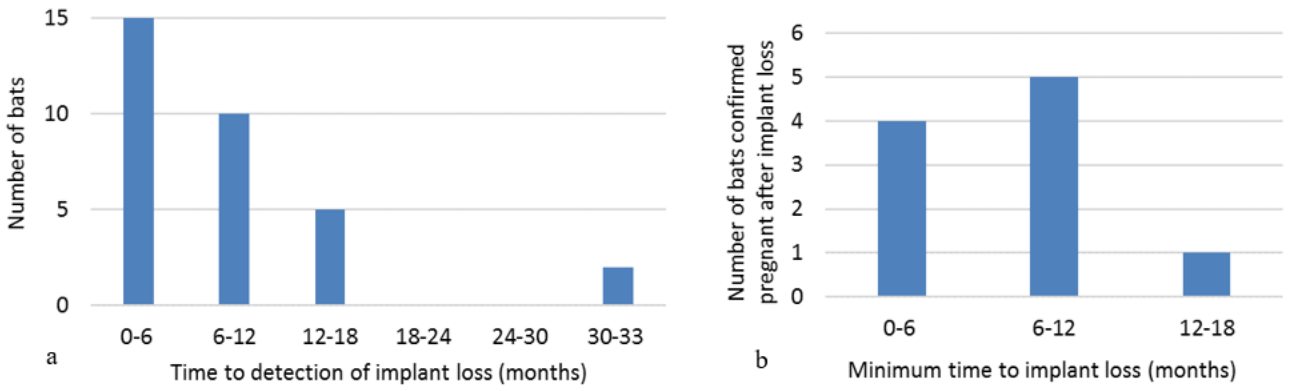

Figure 4. Etonogestrel implant losses in Rodrigues fruit bats; timeframes from implantation to implant losses, and number of Rodrigues fruit bats with lost implants confirmed pregnant after various implant durations. Figure 4a. Time to detection of implant loss in Rodrigues fruit bats. Implants were present at prior censuses. Figure 4b. Number of Rodrigues fruit bats with lost implants confirmed pregnant and minimum duration of implant action prior to loss.

Table 2. Number of etonogestrel and aglepristone treatments given, number of etonogestrel implants lost and total number of individual Rodrigues fruit bats contracepted with etonogestrel at each census. ^Four bats lost their implants during the six weeks isolation after implanting and were replaced without access to males, so count as implants lost, but not as additional bouts of contraception. *Three bats were contracepted during pilot work in March 2017.

Census Date	Aglepristone-treated bats	Bats receiving their first etonogestrel-implant	Bats with lost and replaced etonogestrel implants	Total etonogestrel -contracepted bats to date
Jul 2017	36	42	4^	45*
Mar 2018	37	41	3	86
Oct 2018	27	14	16	100
Mar 2019	0	14	5	114
Oct 2019	0	0	1	114
Mar 2020	0	18	1	132
Oct 2020	0	6	1	138
Total	100	135	31	138

only one bat had reproductive tract histology in this time period.

Since initiation of the contraception management plan in March 2017, 22 sexually mature female bats have died (Supplemental Table 1). Of these 22 female bats, seven were not contracepted. Four non-contracepted bats received gross, and one histological examination of the reproductive tracts; no reproductive abnormalities were detected.

The duration of contraception in the 15 implanted bats ranged between 1 to 39 months at the time of death. Of these 15 implanted bats, four were too autolysed or did not receive a necropsy. Seven had no gross reproductive abnormalities detected. Four bats showed gross reproductive abnormalities. Two had unilateral ovarian cysts, confirmed by histology. One geriatric bat had an incidental uterine leiomyoma. The last was also a geriatric bat and had 1.5 mm vascular dilations of the uterine serosa and superficial myometra adjacent to the ovaries. Histological examination could not attribute any significance to these vascular dilations. Histology was carried out on eight contracepted bats, two (25%) of which had mild or very mild cystic endometrial hyperplasia.

Aglepristone efficacy and safety

A total of 100 paired 4.5 mg doses (range 9.4–21.4 mg/kg) of aglepristone were administered to bats between July 2017 and October 2018. The reproductive status of these bats at administration could be either early pregnant or no detectable pregnancy (an unknown number of which were actually pregnant due to detection failures). Sixteen bats (16%) went on to carry full term pregnancies identified as either very late pregnancy or as lactating females at the next six-month check, eight of which had not been detected at the time of aglepristone treatment.

In 32 cases, aglepristone was given to bats with ultrasound confirmed pregnancies, eight of which (25%) failed to terminate and went on to full term. One pregnancy resulted in the birth of a pup with congenital deformity (patent urachus).

Aglepristone failure (confirmed pregnancies that did not terminate with aglepristone) increased as female age increased (odds ratio (OR) 1.7; P=0.02) and there was a tendency for aglepristone failure to decrease with increased pregnancy size (mm), but this was not significant (OR=1.7; P=0.07). Dose (as it relates to female weight) was not significant.

No side effects attributable to aglepristone administration were noted in the female bats either at the time of administration or on subsequent examinations.

Discussion and conclusions

Etonogestrel implants (34 mg dose) placed subcutaneously proved 100% effective at preventing pregnancy for up to 36 months in sexually mature female Rodrigues fruit bats, making this a good long-term option for contraception and population management of this species. For the first time, in 2020, the natural (non-cull) mortality exceeded the birth rate for the colony. The slight increase in colony size seen in 2019 was due to the large recruitment of young breeding females in that year and to the altered contraception methodology, with cessation of aglepristone use, and implantation of pregnant females, thus still producing a pup the year they were contracepted. At present growth and mortality rates, with 85–90% of females contracepted and only 10–15 females breeding annually, or a birth rate per sexually mature female of ~0.13/year, the colony can be managed down to <175 bats over the next 5–6 years. The reduced stocking density should reduce the risk of iniosis and any other problems associated with overcrowding.

Recent evidence suggests a five or even six-year efficacy of etonogestrel implants in women (Ribeiro 2018). In order to further examine the duration of etonogestrel contraception for this species and to minimise costs and additional procedures on the bats, a proportion of implants will be left in place until there is a natural reversal of contraception. Moreover, some implants will be removed after three years if no natural reversal (i.e., implant failure) occurs, and other, non-implanted bats will be contracepted in their place in order to maintain genetic diversity in the colony and to give different individuals the opportunity to breed. As this programme has shown no evidence of progesterone contraception during pregnancy affecting parturition in this species, there is flexibility on which females can be implanted at any given census, making this strategy easy to achieve.

Etonogestrel implants proved extremely easy to administer. However, a very significant difference in implant loss rate was seen between the two locations used. Initially, the medial brachium was chosen for ease of removal and visibility for follow up checks as per the RMG guidelines (van Bolhuis 2017). However, there was a 34.8% rate of loss over the four years reported from this location, compared to just 1.3%, or one implant, from the inter-scapula location over two years. Loss from the brachial location did not occur immediately after insertion, that is, through the insertion hole, and in 17 of 31 cases, implant loss occurred after

at least 6 months. The skin of the brachium is much thinner and the action of the wing musculature during flying may serve to force the rods out through the skin over time. No evidence of self-trauma was found in any bat and two bats were even found with the rod partially protruding through the skin of the brachium six months and two and a half years after insertion. Not only does implant loss risk unwanted pregnancy, but it is also costly, as each dose costs approximately £37.50. However, if implants remain in place and efficacious for at least three years, potentially longer, and can be placed without an anaesthetic, as in the inter-scapula position, the technique is more cost effective. Placement subcutaneously between the scapulae is strongly advised for this species/taxon. Implants remain palpable under the skin in this location and the authors believe removal will be simple under a general anaesthetic. Implant location did not affect efficacy.

Although this programme did not seek to directly assess reversibility, pregnancy among the bats that lost implants while in the main colony suggest a minimum reversal rate of 34.5%. It is unknown at what stage in the intervening six or nine months between censuses that a bat lost the implant, and therefore whether she would have had time to cycle and become detectably pregnant after the loss. Similarly, the apparent low pregnancy rates likely reflect the short time from implant loss to replacement, which could be a maximum of six or nine months and potentially less. Generally, contraception with synthetic progestogens is considered to be one of the most easily and reliably reversible contraceptives (Ruddick 2009), and removal would be expected to give a swift return to fertility; early data are promising that this is the case.

Contracepted female bats increased their body weight by 21.4% in the year after implant insertion, a side effect of etonogestrel also described in humans (Merck 2020). Weight gain could be a side effect of the raised progestogen levels or reduced metabolic demands in the absence of pregnancy and lactation. This was a significant weight gain and represents a negative health impact of etonogestrel implants on the bats. Obesity is associated with increased risk of cardiovascular, endocrine, neoplastic, musculoskeletal, hepatic and renal disease in humans (Pi-Sunyer 2009) and some domestic species (Sandøe 2014; German, 2018) and this may also be the case for pteropid bats. Longer-term data is required to assess the impact of this on the bat population's health. A pre-emptive reduction in diet quantity is advised when using progestogen contraception but is challenging in a large colony where competition for food and dominance can be a problem. Feeding strategies to encourage flight within the exhibit may also be beneficial to prevent weight gain.

Two histopathological lesions, potentially attributable to extended levels of progestogens, were identified, ovarian cysts and mild uterine endometrial hyperplasia. In suids and tayasuids, use of progesterone-based contraception is associated with increased risk of ovarian cysts (Goblet 2019). Moreover, synthetic progestogen use is a risk factor for endometrial hyperplasia, hydrometra, fibrosis and adenomyosis (Moresco 2009) and pyometra (Asa 2014) in canids, whilst in felids, it has been associated with malignant mammary neoplasia (McAloose 2007). In squirrel monkeys *Saimiri sciureus* and Goeldi's marmosets *Callimico goeldii* progestogen MGA implants, but not etonogestrel implants, have been associated with severe uterine lesions and MGA implant use in these species was subsequently discouraged (Murnane 1996). Here, no reproductive abnormalities were found in non-contracepted females while only one implanted female had minor ovarian cysts, one had mild uterine endometrial hyperplasia and one bat had both lesions. The ovarian cysts could be the consequence of sustained progestogen exposure, a non-regressed follicle that became cystic, or it could be natural. With their small size, spontaneous regression might still be expected, as in humans using etonogestrel implants (Merck 2000), or regression on removal of the progestogen stimulus. This is another reason to encourage rotation of contraception in population management strategies. The severity of the endometrial hyperplasia noted in the two bats was mild and judged to be reversible. However, longer-term, this lesion could progress to irreversible reproductive disease. Given only one adult female bat had uterine histology before the start of the contraception programme and one non-contracepted female since then, it is possible these changes are not uncommon in this taxon. Further data are required to examine the incidence of cystic ovaries and endometrial hyperplasia in etonogestrel implanted bats and any longer-term effects on reproductive success.

The reproductive lesions identified could be dose dependent. The 34 mg dose (1/2 an implant, mean 112.9 mg/kg) used in these bats is relatively high when compared with other species such as great apes where 68 mg (1 implant, 1.2–1.5 mg/kg) is used, or species closer in body weight, such as callitrichids or rodents where 17 mg (1/4 implant) is used (AZA RMC, EAZA RMG 2020). Despite high background progesterone in this species (Crichton 2000), and reports of implant failures at lower doses, it may be warranted in the long term to try lower doses by reducing implant size to 23 mg or 1/3 of an implant and monitoring for implant failures and occurrence of reproductive tract lesions. Reduced doses may also reduce weight gain associated with etonogestrel use.

Upon maturation, one female bat, whose dam failed to terminate pregnancy with aglepristone and was etonogestrel implanted during pregnancy showed a degree of masculinisation of the external genitalia. This is a possible side effect reported for etonogestrel where animal studies have shown that very high doses of progestagenic substances may cause masculinisation of female foetuses (Merck 2020). It is possible progestogen doses here, combined with naturally high background progesterone levels in bats, could have had this effect, but equally, that it is a natural spontaneous phenotypic variation. No other cases of genital masculinisation were noted. During the programme, a bat was born with a congenital deformity, patent urachus, to a female treated with aglepristone which failed to terminate the pregnancy. No evidence that aglepristone or progesterone levels are involved in development of patent urachus could be found in the literature. It is unlikely, although still possible, this was related to the use of aglepristone. No further congenital abnormalities were noted.

The 25% failure rate of aglepristone in the bats was high when compared to other species including domestic (bitches, queens, rabbits, rats: Gogny 2016; sheep: Ozalp 2018; cattle: Breukelman 2005) and exotic species (African wild dogs and African lions: Bertschinger 2016), in which near 100% efficacy is reported with similar or lower doses, and, in some cases, even when only administered once. The relative resistance of the pteropid bats to the effects of aglepristone may be due to insufficient dose in the light of high background circulating progesterone levels. However, as the age of the female treated had a significant effect on pregnancy termination, but dose did not, older females may have a higher or stronger hormone drive. Interestingly, the size of pregnancy had an effect on aglepristone efficacy with a tendency for larger pregnancies to abort more reliably. In grey-headed flying foxes *Pteropus poliocephalus* progesterone has been shown to only increase later in pregnancy once the placenta has formed, not while a pregnancy is dependent on the corpus luteum alone (Crichton 2000). If similar hormonal control of pregnancy is seen in the Rodrigues fruit bat, then aglepristone might not be expected to be as successful at terminating early pregnancies, as was the case here. In addition, it is unknown whether Rodrigues fruit bats employ any of the delayed reproductive strategies seen or suspected in other pteropid bats, such as sperm storage (long-tongued fruit bats *Macroglossus minimus*; Crichton 2000), delayed

implantation (straw-coloured fruit bats *Eidolon helvum*; lesser short-nosed fruit bats *Cynopterus brachyotis*; minute fruit bats *C. minutus*; Crichton 2000) or delayed development (Philippine pygmy fruit bat *Haplonycteris fischeri*: Crichton 2000; Seba's short-tailed fruit bat *Carollia perspicillata*: Rasweiler 1997; Indian short-nosed fruit bat *Cynopterus sphinx*: Meenakumari 2005). Any one of these reproductive strategies could impair the efficacy of aglepristone on pregnancy termination. Finally, if aglepristone is administered too soon after mating it may not be effective (Gogny 2016). Mating dates are unknown for the bats, which may also have contributed to the lower efficacy seen.

In conclusion, 34 mg of etonogestrel (68 mg implants, halved) placed subcutaneously were 100% effective at preventing pregnancy for up to three years in Rodrigues fruit bats. Implants placed subcutaneously over the dorsum show very low loss rates and remain easily detectable. Reversal of contraceptive effects after implant loss is possible, with confirmed pregnancies in some cases. Consideration should be given to lowering the dose of etonogestrel in these bats as secondary effects of significant weight gain were observed, with uncertain long-term health implications. Possible secondary reproductive lesions associated with high doses and or prolonged progestogen exposure, such as ovarian cysts and endometrial hyperplasia, were also identified, although more and longer-term data are required to clarify if this is a true effect. Aglepristone for termination of pregnancy was 75% effective. Data would suggest that delaying use until pregnancies are more established and that higher doses for older bats might have better efficacy. No side effects were noted from aglepristone use. Etonogestrel implants and aglepristone can be successfully used as part of an active reproductive management strategy for maintaining colony size and genetic diversity in captive Rodrigues fruit bats.

References

Asa C.S., Bauman K.L., Devery S., Zordan M., Camilo G.R., Boutelle S., Moresco A. (2014) Factors Associated With Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility. *Zoo Biology* 33: 8–19.

AZA RMC, EAZA RMG (2020) Contraception Database. Data accessed 02 December 2020.

Bertschinger H., Caldwell P. (2016) Fertility suppression of some wildlife species in southern Africa-a review. *Reproduction in Domestic Animals* 51: 18–24.

Breukelman S.P., Szenci O., Beckers J., Kindahl H., Mulder E.J.H., Jonker F.H., van der Weijden B., Revy D., Pogany K., Sulon J., Némedi I., Taverne M.A.M. (2005) Ultrasonographic appearance of the conceptus, fetal heart rate and profiles of pregnancy-associated glycoproteins (PAG) and prostaglandin F2α-metabolite (PGF2α-metabolite) after induction of fetal death with aglepristone during early gestation in cattle. *Theriogenology* 64(4): 917–933.

Crichton E.G., Krutzsch P.H. (2000). *Reproductive biology of Bats.* London, UK: Academic Press.

German A.J., Woods G., Holden S.L., Brennan L., Burke C. (2018) Dangerous trends in pet obesity. *Veterinary Record* 182(1): 25.

Goblet C.C., Newell-Fugate A.E., Moresco A., Garner M.M., Agnew D.W. (2019) Retrospective characterization of reproductive tract lesions in relation to age, parity, and contraception in captive suidae and tayassuidae. *Theriogenology* 127: 137–144.

Gogny A., Fieni F. (2016) Aglepristone: A Review on Its Clinical Use in Animals. Theriogenology 85: 555–566.

Lafortune M., Canapp S.O., Heard D., Farina L.L. (2004) A vasectomy technique for Egyptian fruit bats (*Rousettus aegyptiacus*). *Journal of Zoo and Wildlife Medicine* 35(1): 104–106.

McAloose D., Munson L., Naydan D.K. (2007) Histologic Features of Mammary Carcinomas in Zoo Felids Treated with Melengestrol Acetate (MGA) Contraceptives. *Veterinary Pathology* 44(3): 320–326.

Meenakumari K. J., & Krishna A. (2005) Delayed embryonic development in the Indian short-nosed fruit bat, *Cynopterus sphinx. Zoology* 108(2): 131–140.

Melville D.F., O'Brien G.M., Crichton E.G., Theilemann P., McKinnon A., Johnston S.D. (2012) Reproductive seasonality and the effect of the GnRH agonist deslorelin as a contraceptive in captive male Black Flying-foxes (*Pteropus alecto*). *Theriogenology* 77: 652–661.

Merck, Sharp & Dohme Limited 2020. Summary of Product Characteristics - Nexplanon 68mg implant for subdermal use. https://www.medicines.org.uk/emc/product/5720/smpc. Downloaded on 01 July 2020.

Metrione L.C., Verstegen J.P., Heard D.J., LeBlanc D., Walsh A.L., Penfold L.M. (2008) Preliminary evaluation of deslorelin, a GnRH agonist for contraception of the captive variable flying fox *Pteropus hypomelanus. Contraception* 78: 336–345.

Moresco A., Munson L., Gardner I.A. (2009) Naturally Occurring and Melengestrol Acetate-associated Reproductive Tract Lesions in Zoo Canids. *Veterinary Pathology* 46(6): 1117–1128.

Murnane R., Zdziarski J., Walsh T., Kinsel M., Meehan T., Kovarik P., Briggs M., Raverty S.A., Phillips, L. (1996), Melengestrol Acetate-Induced Exuberant Endometrial Decidualization in Goeldi's Marmosets (*Callimico goeldii*) and Squirrel Monkeys (*Saimiri sciureus*). *Journal of Zoo and Wildlife Medicine* 27(3): 315–324.

Mylniczenko N.D., Fredholm D.V., Pye G.W., Freeman H.D., Wheaton C.J., Hall N. (2018) GnRH vaccine reaction in large flying (*Pteropus vampyrus*) In: *Proceedings of the joint EAZWV/AAZV/Leibniz-IZW Conference Proceedings* p.248.

Özalp R.G., Yavuz A., Orman A., Seker I., Udum Küçükşen D., Rişvanli A., Demiral Ö.O., Wehrend A. (2016) Parturition induction in ewes by a progesterone receptor blocker, aglepristone, and subsequent neonatal survival: Preliminary results. *Theriogenology* 87: 141–147.

Pi-Sunyer X. (2009). The medical risks of obesity. *Postgraduate Medicine* 121(6): 21–33.

Prud'homme Y., Ferrell S.T., Couture É.L., Marquet B., Desmarchelier M. (2020) Surgical orchiectomy in fruit bats – Description of two techniques in the Ruwenzori long-haired fruit bat (*Rousettus lanosus*) and the Jamaican fruit-eating bat (*Artibeus jamaicensis*). *Journal of Zoo and Wildlife Medicine* 51(2): 297–302.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rasweiler J., Badwaik N.K. (1997) Delayed development in the short-tailed fruit bat, *Carollia perspicillata. Reproduction* 109(1): 7–20.

Ribeiro B.C., Nogueira-Silva C., Afonso H., Silva P.O., Reis I.D. (2018) Use of etonogestrel implant beyond approved duration: prolonged contraceptive effectiveness. *The European journal of contraception & reproductive health care : the official journal of the European Society of Contraception* 23(4): 309–10.

Sandøe P., Palmer C., Corr S., Astrup A., Bjørnvad C.R. (2014) Canine and feline obesity: a One Health perspective. *Veterinary Record* 175: 610–616.

Tatayah V., Jhangeer-Khan R., Bégué J.A., Jones C.A. (2017) *Pteropus rodricensis.* The IUCN Red List of Threatened Species 2017: e.T18755A22087057. https://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T18755A22087057.en. Downloaded on 01 July 2020.

van Bolhuis H., Strike T. (2017) EGZAC Guidelines – bats. https://www.egzac.org/home/viewdocument?filename=EGZAC%20Chiroptera%20taxon%20sheet%202017.pdf Downloaded on 22 February 2021.

Virbac Limited (2009). Alizin 30 mg/ml solution for injection. NOAH Compendium 2020 (http://www.noahcompendium.co.uk). Accessed: Thursday, July 2, 2020 9: 11.