Manaaki Whenua
Landcare Research

Prospects for manipulating embryonic diapause as a method for stoat fertility control

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Prospects for manipulating embryonic diapause as a method for stoat fertility control

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Summary

Project and client

- Predator Free 2050 Limited is a Crown-owned, charitable company established to help deliver the New Zealand government's ambitious goal of eradicating possums, stoats and rats by 2050 (Predator Free 2050).
- Manaaki Whenua – Landcare Research (MWLR) was contracted by Predator Free 2050 Limited to investigate the prospects of manipulating embryonic diapause as a fertility control technique for stoats.

Objectives

- To explore the potential for manipulating embryonic diapause as a fertility control technique for stoats. This includes finding a dosing regime and time of year that can successfully alter diapause, either causing precocious implantation or rejection of the embryos.

Background

- Embryonic diapause is a temporary suspended state of pregnancy where a blastocyst (an early-stage embryo) remains in a suspended state of development that does not implant in the mother's uterus. This dormant state is maintained until externally induced hormonal changes cause the blastocyst to implant and proceed to a full pregnancy.
- All stoat (*Mustela erminea*) pregnancies begin with an obligate c. 9-month period of diapause, synchronising pregnancy to an annual cycle.
- Across mammals with embryonic diapause, the hormonal regulation of diapause is complex, generally beginning with environmental cues around photoperiod resulting in changes in hormones such as melatonin. These changes then lead to further expression-level changes in hormones including prolactin, progesterone, oestrogen, leptin, and insulin-like growth factor.
- In American mink (*Neogale vison*), a species related to stoats, there is a shorter, facultative period of diapause. Previous research has shown that in this species, manipulating prolactin alone is enough to manipulate diapause, with increased prolactin capable of inducing implantation of the blastocysts.
- It has been speculated that altering prolactin levels in stoats could also regulate exit from diapause, making it a potential fertility control method for this species.

Methods

- We obtained 39 female stoats over two seasons which had all been mated in the wild and contained fertilised blastocysts.
- We initially determined whether pimozide would increase prolactin secretion in stoats through injecting a subset of animals and then monitoring serum prolactin using a canine prolactin ELISA.
- For the first trial we examined the effect of orally dosing female stoats (in March) with either pimozide, which is known to dramatically increase prolactin secretion, or with cabergoline, which decreases prolactin levels.
- In a second trial, which was part of a larger trial investigating the genetic regulation of embryonic diapause over the year, we injected female stoats with pimozide in early September, with the hypothesis that this would induce a slightly earlier implantation that was synchronised between individuals.
- For both trials we dissected out the uteri and counted and measured the blastocysts and recorded whether implantation had occurred.
- All tissues and blastocysts were rapidly frozen for transcriptomic (gene expression) analyses.

Results

- We demonstrated that pimozide does significantly increase prolactin in stoats.
- With both the dosing schedules (orally in March, or by injection in September) we were unable to manipulate embryonic diapause with either pimozide or cabergoline (i.e. the number, size and state of blastocysts was unchanged by interventions).
- Further analysis of the transcriptomic data will need to be completed to understand what effects (if any) the manipulation of prolactin levels had on the physiology of the animals used within the trial.

Conclusions

- Either higher and/or more prolonged dosages for pimozide or cabergoline than trialled will be required to manipulate embryonic diapause in stoats, or manipulations of the prolactin pathway are insufficient on their own to affect diapause.
- If diapause can be manipulated as a fertility control mechanism in stoats the most likely pathway remains using oral doses of pimozide at higher and/or more repeated doses than those trialled.
- Cabergoline may still have potential as a fertility control tool for stoats. However this would not be through it manipulating diapause but more probably through it acting an abortifacient, and through its inhibitory action on lactation.

Recommendations

- Conduct further experiments with higher/repeated doses of pimozide or prolactin itself on female stoats around March/April. These may still yield a dosing regimen that would confirm or deny the possibility of manipulating this pathway in isolation as a fertility control method.
- If pimozide can induce precocious implantation in stoats at higher doses, conduct additional research to demonstrate that if implantation occurs then any litters are aborted (unlike in mink where pregnancies go to full term and are successful).
- Consider undertaking experiments on the prospects of cabergoline as a fertility control tool (with the dosing during the last 2 weeks of pregnancy). However, the challenges of conducting meaningful experiments of this sort on captive animals means we advise undertaking a cost-benefit analysis of this technique versus progressing toxins such as para-aminopropiophenone (PAPP).

1 Introduction

In New Zealand (NZ) the terrestrial fauna evolved without predatory mammals (Gibbs 2006; Tennyson 2010), resulting in an avian fauna that is often flightless or semi-flighted, slow to mature and breed, and naïve to mammalian predation. Since the arrival of humans around 1280 AD, approximately 40% of bird species have become extinct, (Worthy & Holdaway 2002). Among the extant native avian taxa about 80% are currently classified (in the New Zealand Threat Classification System) as Threatened or At Risk (Robertson et al. 2017), which is one of the highest percentages in the world (Clout 2001). Invasive mammals were, and continue to be, the primary agents of decline for most of these birds (Parkes & Murphy 2003; Innes et al. 2010).

In 2016, the New Zealand Government set the ambitious goal of eradicating the principal invasive predatory mammals by 2050 – Predator Free 2020 (PF2050, hereafter). These species include three mustelids: ferrets (*Mustela putorius furo*), stoats (*M. erminea*) and weasels (*M. nivalis*); three rats: ship rats (*Rattus rattus*), Norway rats (*R. norvegicus*) and kiore (*R. exulans*), and the brush-tailed possum (*Trichosurus vulpecula*) (Russell et al. 2015; Owens 2017). The eradication of invasive predators at this country-wide scale has never been attempted and is widely considered impossible with the traditional toolkit. Therefore, we require significant technological, operational, and social advances if it is to succeed (Owens 2017; Tompkins 2018; Murphy et al. 2019; Peltzer et al. 2019; Ross et al. 2020).

It appears impossible that manual methods such as trapping or shooting can be scaled up to the large landscape scales required for the nationwide eradication of any of these species. It is debatable that even small-scale eradication using these methods is achievable, given this has never been demonstrated. The current alternative to trapping and shooting is the application of toxins (which can be successful at smaller scales on islands or in fenced sanctuaries). However, the available toxins (e.g. brodifacoum and 1080 [sodium fluoroacetate]) are broad spectrum, meaning that there are significant risks to non-target species. The available toxins also have high welfare impacts for the poisoned animals, causing ethical concerns (Nguyen et al. 2022; Warburton et al. 2022). Understandably, there is significant social concern and resistance to the widespread use of these toxins across the landscape. The conundrum then for PF2050 is that the tools that may work do not have social licence, and the tools that do have social licence will not work for eradication.

1.1 Potential fertility control options

One possible research area with the potential to produce 'game changing' advances for invasive species eradication is fertility control (Harvey-Samuel et al. 2017; Tompkins 2018; Jacoblinnert et al. 2022; Croft & Massei 2023). Potentially, fertility control can be targeted to be species-specific, thus avoiding non-target impacts, with the added advantage that fertility control has lower welfare impacts for the target animals. Many people feel the better welfare outcomes of fertility control make it preferable to lethal control (Oogjes 1997; Rutberg 2013; Gamborg et al. 2020). While the method of application will significantly affect the social licence to use a fertility control tool (Kirk et al. 2019), it appears likely that some methods of fertility control may be capable of obtaining a

broader social licence than the other currently available tools. For stoats, if fertility control can be developed successfully it is likely that it could be as effective as lethal control (Barlow & Barron 2005).

Of the pests targeted for eradication by Predator Free 2050, stoats uniquely exhibit a reproductive strategy known as 'embryonic diapause'. In embryonic diapause, each blastocyst (an early-stage embryo) remains in a suspended state of development that does not implant in the mother's uterus. This dormant state is maintained until externally induced hormonal changes cause the blastocyst to implant and proceed into a full pregnancy. Embryonic diapause is a common reproductive strategy among a diverse range of mammals, and its regulation is complex with diverse mechanisms, though there are commonalities among species (Fenelon et al. 2014; Fenelon & Renfree 2018).

Diapause can be facultative, i.e. induced by physiological conditions, or obligate, i.e. present in every gestation of a species. In the latter case, the proximal signals for regulation are related to photoperiod. Across the carnivores with embryonic diapause that have been studied to date, prolactin appears to be the principal driver of the luteal reactivation that is needed to make the endometrium receptive to implantation of the embryo; although other ovarian steroids (particularly progesterone) also play important roles (Fenelon et al. 2017a).

Stoats exhibit a long, obligate period of diapause, where the embryo is paused as a blastocyst for around 9 months, one of the longest recorded periods among mammals (Deanesley 1935; King & Moody 1982; Fenelon et al. 2017a). Juvenile female stoats (along with their mother) mate in spring shortly after they are born. The fertilised blastocysts are then maintained in a dormant (diapaused) state in the uterus until a series of hormonal changes occur, which are activated by a change in photoperiod during winter, causing the fertilized embryo to implant and begin a full pregnancy. This adaptation enables stoats to time births to align with favourable environmental conditions. Manipulating embryonic diapause in stoats is an attractive prospect for conservation control programmes in New Zealand, given: (i) the methods used may be highly species specific; (ii) there is a 9-month window in which any interventions could be introduced to eliminate the litters.

Embryonic diapause is a highly regulated process, with environmental factors such as photoperiod and temperature influencing endocrine regulation. In mustelids the initiation and termination of diapause are governed by a range of hormonal pathways, with prolactin playing a crucial role in triggering implantation (Fenelon et al. 2017a, 2017b). Previous research on the mechanisms underpinning embryonic diapause in the related mustelid, the American mink (*Neogale vison*), has shown that in this species, prolactin is the only gonadotrophin required to regulate entry into, maintenance of, and exit from diapause. High prolactin levels induce the blastocyst to implant, leaving diapause and administering prolactin alone can induce precocious implantation (Murphy et al. 1990; Marks et al. 2006). Lower levels of prolactin are likely to promote diapause, and a low to moderate concentration of prolactin may be required to maintain the blastocyst in the uterus. For mink, when artificially elevated prolactin causes the precocious implantation of the embryos, a full normal healthy litter can still be born (Marks et al. 2006). Activation of the embryo from diapause in the mink begins first with embryo expansion, within 24 hr of

prolactin treatment of the animal, followed by rapid protein synthesis over the next 48 hr (Desmarais et al. 2004).

Mink exhibit a shorter facultative diapause that is induced by physiological conditions, compared to the long obligate period of diapause found in stoats. These differences between stoats and minks may mean that the mechanisms regulating diapause may differ between these species, and the outcomes of manipulating diapause may also differ. Marks et al. 2006 speculated that if diapause in stoats could be terminated during the short days of winter, the embryos might not prove to be viable. This is because maintaining a pregnancy typically requires higher levels of progesterone, which is produced by the corpus luteum. This process is supported by sustained elevated prolactin, which in turn is influenced by seasonal changes in melatonin concentrations triggered by photic cues. Marks et al. (2006) proposed that in stoats, a sudden surge in prolactin may trigger embryo implantation. However, without consistently elevated prolactin levels, along with the typical seasonal levels of hormones like progesterone and melatonin, these implanted embryos may be aborted.

1.2 Chemicals affecting diapause and fertility

There are multiple chemicals known to regulate prolactin in mammals that could potentially be used to regulate embryonic diapause. Pimozide, a dopamine antagonist, causes the hypersecretion of prolactin, terminating diapause, and inducing early implantation in the mink (Murphy 1983). Pimozide, delivered both sub-cutaneously or orally, has been successful in inducing precocious implantation in mink (Marks et al. 2006). In contrast, cabergoline is a dopamine agonist, which suppresses prolactin secretion by stimulating dopamine receptors in the pituitary gland. This decrease in prolactin results in the regression of the corpus luteum and a decrease in progesterone production, preventing or terminating pregnancy in some mammals (Onclin et al. 1995; Negishi & Koide 1997). Another drug, bromocriptine, works in a similar manner to cabergoline but has a longer established use in reproductive research, including in embryonic diapause. However, cabergoline is more likely to be orally tolerated and has a longer half-life, therefore it is more likely to be effective for bait delivered applications. Given that a practical wildlife control agent must be easily administered via bait, oral effectiveness is a key consideration for population control strategies.

All these drugs offer a promising means of manipulating reproductive timing and fertility in stoats. Pimozide may (as in the mink) induce precocious implantation, but if administered early enough in the year may (unlike in mink) cause these pregnancies to be non-viable. Cabergoline could potentially lower prolactin levels sufficiently to cause the loss of the blastocysts during diapause; or it could potentially be used for terminating the pregnancy if implantation does occur.

Our study aimed to investigate whether manipulating the prolactin pathway can terminate embryonic diapause in stoats, potentially providing a novel fertility control method. By exploring the pharmacological effects of these agents in stoats, we hoped to gain insights into the feasibility of using hormone-modulating drugs for population management.

2 Objectives

To explore the potential for manipulating embryonic diapause as a fertility control technique for stoats. This includes finding a dosing regime and time of year that can successfully alter diapause, either causing precocious implantation or rejection of the embryos.

3 Methods

3.1 Stoat handing and husbandry

For these experiments all stoats were wild-caught animals sourced from Haast, South Island, NZ. We obtained a total of 39 female stoats for the research. All animals were held in individual pens and under natural light conditions.

This study was conducted in accordance with the animal ethics approval granted by Manaaki Whenua – Landcare Research Animal Ethics Committee with the ethics approval number 221103.

3.2 Determining whether injected pimozide elevates prolactin in stoats

We used a canine prolactin ELISA kit (MyBioSource Canine PRL (Prolactin) ELISA Kit, MBS7608106) to confirm whether the proposed pimozide dosing caused an increase in prolactin levels in stoats. This study was undertaken in May, the time of year when prolactin levels are naturally low in stoats.

We used three non-pregnant female stoats (that had been isolated from other stoats in the animal facility for over a year to ensure they could not become pregnant) for this experiment. Stoats were first anaesthetised by inhalation of isoflurane anaesthetic. They were then injected subcutaneously on 5 different days (Days 0, 3, 6, 9 and 11) with 2mg/mL pimozide dissolved in 1:20 glacial acetic acid and Milli-Q® water (according to the ELISA kit protocol). Each dose of pimozide received per each individual were 0.2, 0.3 or 0.4 mg/kg, Blood samples were taken on Days 0 (pre injection), 9, and at euthanasia on Day 17 (PM). A fourth female stoat was used as a control and euthanised without dosing.

We undertook an ELISA test run with PM serum diluted 1:1 in dilution buffer, but a comparison against the kit's standard curve showed plasma samples needed to be used undiluted. Plasma samples from a single male and single female stoat euthanised in May were used as our untreated controls.

Since stoat prolactin levels are typically low during this period (May), we did not confirm directly whether cabergoline and bromocriptine further reduced prolactin levels. Instead, we assumed, based on observations in related species, that these treatments would lower prolactin even more.

3.3 Trial 1: oral dosing of pimozide and cabergoline

A total of 12 stoats were dosed in late April/early May. Stoats were anaesthetised by inhalation of isoflurane anaesthetic. Once the stoat was unconscious a 18G flexible gavage tube with a silicon bulb end was directed into the stoat's mouth and guided into its oesophagus. Once the tube was inserted to a predetermined mark (measured to ensure the needle reached the bottom of its ribs where the stomach lies) the solution was administered, and the gavage tube removed.

For both the pimozide and the cabergoline, solutions were made up by dissolving the solid pure chemical in Dimethylsulfoxide (DMSO) then making the solution up in 0.2M HCl to 5%DMSO. Female stoats were randomly assigned into one of three groups: 1) cabergoline (6 stoats), pimozide (6 stoats), control (3 stoats). For cabergoline, two doses were given to each stoat, spaced 3 days apart, with each dose containing 50 µg/mL at 50 µg/kg of body weight. For pimozide, a single dose was administered using a 0.78 mg/mL solution at 1.6 mg/kg of body weight. After the dose was administered each stoat was held upright until it was observed to be swallowing and starting to regain muscle tone before it was returned to its nest box. Dosed animals were then euthanised 2–4 weeks after their last oral dose as described in the approved Animal Ethics Committee application (see Section 3.1.1).

Each stoat was dissected immediately after death, and the uterus removed and flushed to isolate the blastocysts. Both horns of the uterus were examined to ensure all blastocysts and embryos were recorded and measured. All tissues and blastocysts were then stored at –80 for RNA extraction.

3.4 Trial 2: wider study of subcutaneous injection of pimozide

This part of the project was part of a larger study investigating the genetic regulation of embryonic diapause. Throughout the year at various time points, female stoats were euthanised, and their endometrial tissue and blastocysts were frozen for transcriptional analyses. To synchronise embryonic diapause, we administered subcutaneous injections of pimozide in early September. This timing was chosen because at Lincoln's latitude, it represents an early, but not too early implantation widow, meaning other hormonal cues would likely to be close to the levels needed for implantation.

In this part of the experiment, six female stoats were given subcutaneous injections of pimozide prepared according to the ELISA kit protocol (2mg/mL pimozide dissolved in 1:20 glacial acetic acid and Milli-Q® water). The highest dose from the initial experiment described in Section 3.2 (i.e. 0.4 mg/kg) was used, and injections were administered on Days 0, 3, 6, 9 and 11. The experiment began on 5 September (Day 0) with all stoats euthanised on Day 25.

4 Results

We will analyse the transcriptomic results from our samples to determine whether they can add to our understanding and reveal valuable pathways for further investigation. However, those results are not reported upon here.

4.1 Determining whether injected pimozide elevates prolactin in stoats

The canine prolactin ELISA kit worked well for stoats and the standard curve had a high goodness of fit (R^2 = 0.98). Figure 1 shows the results of this experiment.

We were unable to collect sufficient blood to provide a reading from the stoat dosed with 0.2 mg/kg on Day 9, or the one dosed with 0.3 mg/kg on Day 0 (pre-dosing). However, the results still show a substantial increase in prolactin levels across all three dosed stoats. A fourth female stoat used as a control and euthanised without dosing, had a serum prolactin concentration of 0.0 ng/mL.

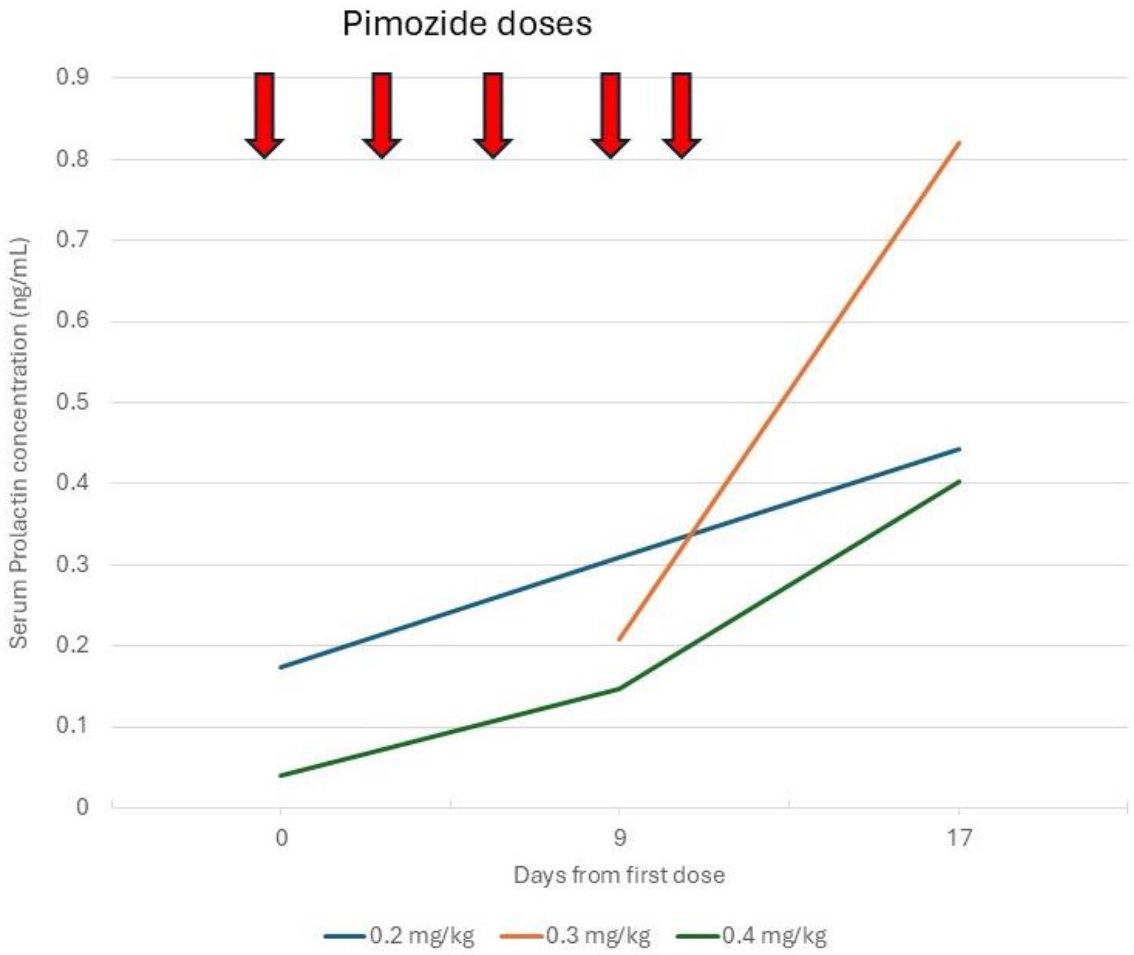

Figure 1. Serum prolactin concentrations for stoats injected with varying doses of pimozide (Red arrows show the 5 days on which dosing occurred; dose rates shown by coloured lines.)

Our results showed that pimozide does increase prolactin levels in stoats at the dosing regimen used, allowing us to move forward with investigating its effect on embryonic diapause. It is implausible that prolactin levels could have increased more than fourfold over 17 days naturally at this time of year. This experiment provided enough evidence to suggest that the dosing used by (Marks et al. 2006) for mink should also be suitable for stoats. While we did not test whether the canine prolactin ELISA is as sensitive to stoat prolactin as it is to canine prolactin, it is highly likely that the relative changes between samples are accurate – even if the absolute serum concentrations measured may not be a true reflection.

4.2 Trial 1: oral dosing of pimozide and cabergoline

We found no evidence of implantation or loss of the blastocysts from either pimozide or cabergoline treatment, nor were there any observed changes in the uteri or blastocysts. The average number of blastocysts for each female was roughly the same across treatments: n = 9.2 for cabergoline, n = 9.5 for control, n = 10.4 for pimozide. All blastocysts were approximately the same size across treatments (mean = 1.1 mm in diameter), and all had a similar appearance. One of the cabergoline-treated stoats had a developmental deformity in which one horn of the uterus was not developed, but this was highly unlikely to be related to the treatment and did not affect the results.

Based on these results neither cabergoline nor pimozide, at the dosages used (when taken orally) caused any significant disruption to diapause in stoats when administered in April/May. However, this does not rule out the possibility that higher dosages could be effective, or that administering these drugs at other times of the year, when hormone levels vary seasonally, might induce implantation or rejection of the blastocysts.

4.3 Trial 2: wider study of subcutaneous injection of pimozide

There was minimal evidence that pimozide injections could induce implantation during September, despite this being close to the natural implantation period. Of the six stoats dosed with pimozide, five had not implanted embryos even after 25 days. However, one female had implanted embryos, that had grown to 7 mm in diameter.

The average number of blastocysts recovered per female from the pimozide-treated stoats was 7.8, comparable to previous treatments. The size of the blastocysts was comparable to those collected from control stoats not treated with pimozide in August. Blastocyst appearance was identical between control and treatment groups.

Since September falls within the natural implantation window for stoats subjected to the ambient light cycle at Lincoln's latitude, it is possible that the single instance of implantation was natural timing, rather than caused by pimozide dosing. The fact that these embryos appeared to only have recently implanted, supports this theory. Additionally, any exposure to artificial light for this individual could also have caused implantation to occur slightly earlier than in the others.

In this subcutaneous injection study we attempted to manipulate embryonic diapause in stoats using drugs that affect prolactin release. Neither treatment was successful in disrupting pregnancy in stoats, suggesting that higher dosages may be needed, the seasonal timing of the treatment needs to be adjusted, or prolactin manipulation alone may not be sufficient to alter the embryonic diapause process in stoats.

5 Discussion

5.1 Pimozide

Currently, we do not have sufficient evidence to show that pimozide alone can manipulate embryonic diapause in stoats. If pimozide can manipulate diapause in stoats, as it does in mink, it would probably require significantly higher or more frequent doses. Based on our results, it seems that prolactin level alone may not be enough to regulate diapause in stoats, and other hormones may need to be simultaneously adjusted to trigger implantation.

If a sufficient dose of pimozide is found there are two possible outcomes: 1) precocious implantation leading to a non-viable pregnancy; 2) successful implantation occurs, resulting in pregnancy and a full litter of kits being born about 4 weeks after dosing.

Marks et al (2006) speculated that the first scenario might occur in stoats if diapause is terminated during short days. This is because melatonin levels influenced by light-related cues and seasonal changes may be necessary to increase progesterone levels to sustain the pregnancy.

5.1.1 Could pimozide be used as a fertility control for stoats?

If a dose of pimozide can be determined that induces non-viable precocious implantation then it could be a successful method for fertility control for stoats. Whilst this remains a possibility, the repeated doses used in our experiments were unsuccessful, suggesting that pimozide may not cause a high enough or sustained prolactin release to trigger implantation. It is also possible prolactin alone is insufficient to induce implantation.

If precocious implantation could be triggered and resulted in a normal pregnancy with kits being born (as observed in mink), stoat populations might still be reduced to some degree, if the correct time of year was chosen for dosing. If litters are born when food is scarce their survival could be significantly affected. The environmental factors that influence rodent abundance are complex, and rodent population patterns and irruptive growth vary across forest types and regions (Walker et al. 2019). If rodent populations are typically low in autumn or early winter, stoat litters born during this period might struggle to survive. However, the ideal outcome would be to induce litters that are naturally aborted.

Male stoats are fertile for approximately 4 months, starting in September in the north of New Zealand and October in the south of New Zealand, though juvenile stoats are not fertile until the following season at 11–12 months old (King & Moody 1982). If stoat litters

are induced to be born between March and June, then female kits and their mother would need to wait until September to mate again, and then survive until the following September to give birth. This could potentially reduce the stoat population to some degree. However, unmated female stoats are believed to remain fertile, and early-born female kits are still likely to mate in the spring. While male stoats born early are unlikely to be fertile by spring, this remains a potential risk that should be investigated if early implantation is successful.

The best route forward for pimozide research might be to conduct in vitro experiments on stoat blastocysts under various hormone treatments to determine whether prolactin alone, or in combination with other hormones, can induce implantation. Once the right dose or hormone mixture is identified, further in vivo testing will be needed to determine if implantation progresses normally or leads to abortion.

5.2 Cabergoline

Using cabergoline to lower prolactin levels sufficiently to cause abortion during diapause was always less likely to succeed compared to increasing prolactin levels to manipulate diapause. This is because prolactin levels are already low during most of the diapause period, so a sustained decrease over a long period would likely to be needed to have an effect. While cabergoline has been used to lower prolactin levels and cause embryo loss in a range of mammals, it has only been applied to implanted pregnancies not to diapaused blastocysts.

Researching cabergoline as a fertility control method for stoats is both challenging and, even if successful, may be difficult to implement. Based on our results and the literature it is unlikely that cabergoline will cause abortion of non-implanted blastocysts. To be effective as a fertility control method cabergoline would need to be delivered either after implantation (during pregnancy) or when the mother is lactating. In this case, cabergoline would act as a standard abortifacient rather than manipulating embryonic diapause per se.

5.2.1 Could cabergoline be used as a fertility control for stoats?

If cabergoline can induce abortion of implanted blastocysts in stoats there are two possible times it could be used for population control (see Figure 2). One option is to give cabergoline during natural pregnancy to induce abortion, as has been shown for dogs (Onclin et al. 1993, 1995; Gobello et al. 2004; Corrada et al. 2006; Parmar et al. 2020), foxes (Marks 2001; Marks et al. 2002), and cats (Ay et al. 2018). However, the timing of dosing is critical as studies on other species show that cabergoline administration only reduces fertility through abortion or litter loss when administered in the second half of pregnancy (Marks et al. 2002).

In stoats the period from implantation to birth (true pregnancy) is approximately 4 weeks. The precise timing of implantation is influenced partially by latitude (which affects day length) with births occurring from around the 24 September in the north of the North,Island to around the 29 October in the south of the South Island (King & Moody 1982). If we assume, as in canids, that the window for causing abortion is only 2 weeks long, then a bait containing sufficient cabergoline to cause an abortion in a single dose

would need to be eaten in a 2-week window in mid-September in the north and in mid- to late-October in the south. Coordinating such a large-scale baiting operation would be logistically challenging, especially since female stoats are notoriously neophobic during this period (King et al. 2009).

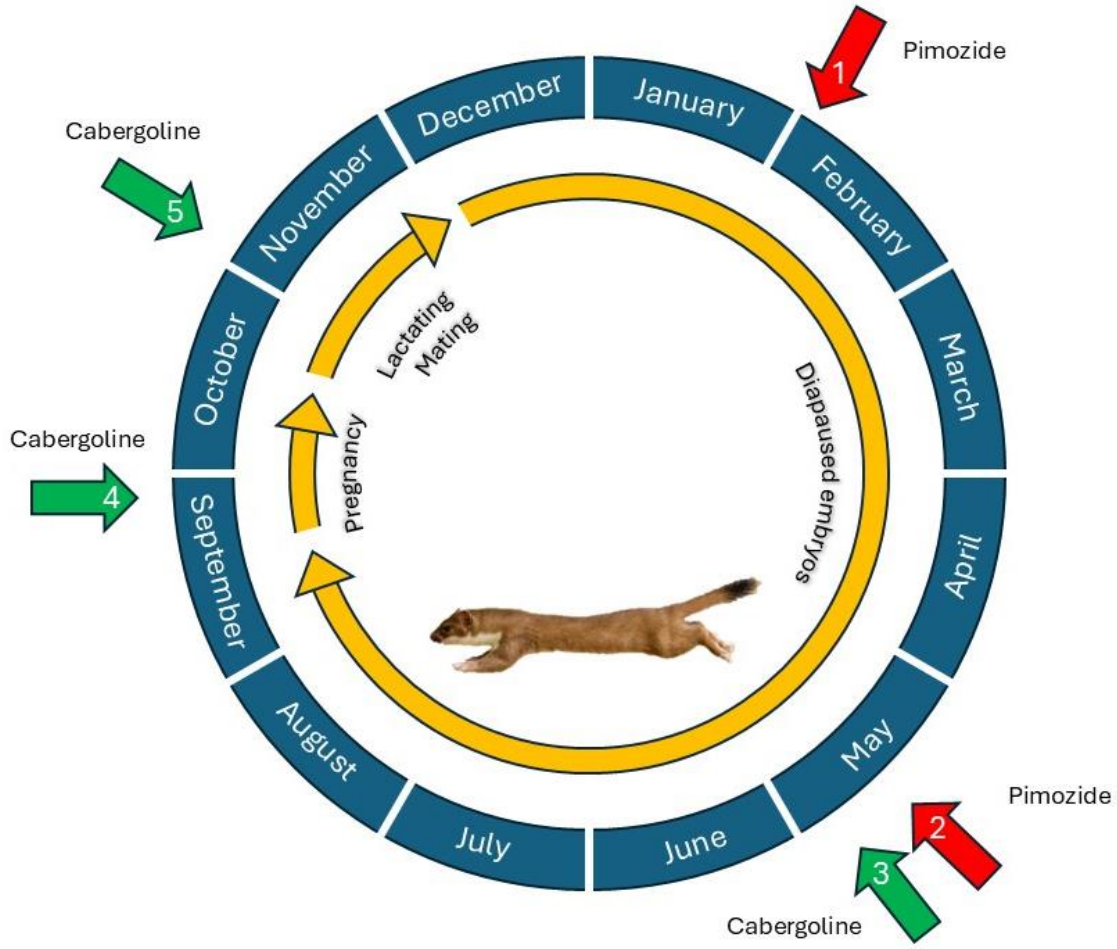

Figure 2. Diagram of the female stoat fertility cycle showing possible future timings (numbered 1–5) for applying prolactin modulators. 1) If a pimozide dose is found to induce implantation the most optimal time for administration would be between February and March, however, it may also be effective as late as May or June. For 2) and 3): if pimozide induces implantation, but pregnancy progresses normally following up with a cabergoline dose 2–3 weeks later may result in abortion of the litter. 4) Depending on latitude, applying cabergoline in the last 2 weeks of pregnancy is likely to cause the litter to be aborted. 5) Cabergoline administered during lactation is likely to cause a stop in lactation causing the litter to be lost.

Alternatively, cabergoline could be used to stop lactation and cause litters to be abandoned. When trialled as a feral cat control method, cabergoline caused mammary gland regression within 36-48 hr, preventing kittens from being nursed, and leading to the kits' rapid demise (Jöchle & Jöchle 1993).

In practice, the most feasible use of cabergoline for stoat control would be an application of cabergoline containing baits during the last 2 weeks of pregnancy to induce abortion. This could be followed by continued baiting to disrupt lactation ensuring that any litters that are born post-partum would die.

5.2.2 Wider considerations and strategies relating to potential cabergoline use in stoat control

While we did not test the use of cabergoline as an abortifacient, or its potential to suppress lactation, it is likely to be effective based on its use in the other related species. The main challenge in progressing research on cabergoline for stoat control is the difficulty of studying it in captive trials since stoats rarely implant blastocysts in captivity, and even when the litter is born, they rarely survive without further manipulation.

A possible research pathway forward would be to determine the dose of cabergoline that reliably causes abortion in ferrets (most probably during the second half of pregnancy). This dose could then be tested on stoats in baits to confirm palatability and to record other side effects. A stoat trial could then be undertaken in the wild with baits hand-laid across a large area during the correct time of year, with a comparative area or areas monitored for juvenile stoat recruitment over the following summer. Given the potential extreme dispersal distances of stoats, the individual treatment areas needed for such a study would likely have to be very large (thousands of hectares) to achieve a measurable outcome.

What potential benefit would such a cabergoline trial have over alternative control methods? If cabergoline baits were proven effective in reducing stoat fertility, the applied mechanism would rely on female stoats consuming the bait to trigger abortions. However, the obvious alternative would be to use baits containing toxins such as 1080, brodifacoum, or para-aminopropiophenone (PAPP). In both instances female stoats must be lured to eat a bait, but toxins can be used at any time of year. A further advantage of toxins is that they kill the female stoat, preventing her from breeding in subsequent years. This comparison makes cabergoline a less appealing option for future research.

A potential *benefit* of using cabergoline over toxins is that it doesn't kill the adult animal, which may make it a more socially acceptable control method. However, some people may still oppose its use, as it causes kits to die of starvation. The risk to non-target species would also likely be lower, but there may still be risk of cabergoline causing abortions in other animals such as dogs, cats or farm stock if they consume a high enough dose.

One concern that would need to be addressed by research is the potential effects of cabergoline on native species, particularly birds. Prolactin is an important hormone in birds, and influences behaviour such as parental care. A recent small study in zebra finches found that cabergoline did not affect prolactin levels, suggesting that this pathway may not work the same way in birds (Smiley & Adkins-Regan 2016). However, if cabergoline is pursued as a stoat fertility treatment, further studies would be needed to assess its effects on a wider range of bird species. These studies would need to determine if it alters prolactin in some groups, and whether this could lead to negative effects such as nest abandonment.

5.2.3 A combined approach?

Another potential strategy for manipulating embryonic diapause, though probably too complex in real world settings depends on: 1) whether a higher dose of pimozide can induce pregnancy; and 2) whether cabergoline can successfully induce abortion. If so then a strategy that involves a two-step process with a combination of pimozide administered between December and June followed by a dose of cabergoline 2–3 weeks later, would eliminate the litter (Figure 2). This complex dosing strategy – requiring a female stoat to ingest two different chemicals within a specific time-interval – seems high unfeasible compared to a single toxin bait, but we mention it here for completeness.

5.3 Manipulating male fertility

While this report specifically focuses on manipulating embryonic diapause for stoat fertility control another option might be to disrupt the *male* stoat's reproductive cycle. Male stoats undergo an annual cycle which regulates reproductive behaviour and tissue growth, with testes dramatically increasing in weight for the breeding season, and sperm production occurring mainly in spring and early summer (King & Moody 1982).

If the testicular development cycle could be disrupted, then population level fertility control could also be achieved. The seasonal development of male stoat reproductive organs is controlled by melatonin which regulates the production of gonadotropin-releasing hormone, follicular stimulating hormone, and luteinising hormone (Li & Zhou 2015). Melatonin may also influence testicular development directly by binding to specific receptors expressed in the testes. Photoperiods also control testicular regression and apoptosis (programmed cell death) (Young & Nelson 2001) probably via melatonin levels. These pathways could provide a hormonal mechanism to disrupt seasonal male sexual development.

Additionally, there are non-endocrine chemicals known to induce sterilisation in male mammals that could also be explored (Hess et al. 2024). Male reproductive control is likely to be easier to study in captivity since it involves measuring testicular development and sperm production rather than trying to get female stoats to implant and carry a pregnancy which rarely happens in captivity).

6 Conclusions and recommendations

We were unable to manipulate embryonic diapause successfully in our trials leaving the potential for using this technique for stoat fertility control undetermined.

In our experiments we attempted to manipulate embryonic diapause by administering pimozide which causes prolactin hypersecretion, and with cabergoline that significantly lowers prolactin levels. This approach was based on the understanding that the prolactin pathway is known to regulate embryonic diapause in related species. However, these manipulations did not induce implantation, resorption, or rejection of the blastocysts.

It is possible that higher and/or more prolonged dosages of pimozide or cabergoline are required to manipulate embryonic diapause in stoats. Alternatively, adjustments to the prolactin pathway alone by either component are insufficient to affect diapause.

If higher doses, particularly of pimozide, can be found to successfully manipulate diapause, this may have applications for fertility control in stoats. The most likely pathway remains using oral doses of pimozide at higher and/or more repeated doses than those trialled. Meanwhile, cabergoline may still be a viable option as a fertility control tool, not through manipulating diapause but potentially as an abortifacient. It may also halt lactation.

6.1 Future steps

The most promising potential pathway forward for exploring fertility control through the manipulation of embryonic diapause in stoats is to continue investigating higher and repeated doses of pimozide, or to use prolactin itself directly.

A key step for progressing research on pimozide would to be conduct in vitro experiments on stoat blastocysts with various hormone treatments. This would help determine whether specific levels of prolactin, or prolactin combined with other hormones can induce implantation. Once that dose or mixture is determined, further dosing in vivo would be necessary to determine if implantation occurs normally or leads to abortion.

Although not specifically targeting embryonic diapause, cabergoline administered to mothers during the second half of pregnancy or shortly after birth for a few weeks may still serve as a fertility control method for stoats. Given the challenges of researching this in pen trials, the most promising way forward is to conduct a cost-benefit analysis of pursuing this method compared to continuing research on bait delivered humane toxins such as PAPP, which has the same delivery mechanisms but is more advanced in development. Research on fatal toxins is generally easier than investigating fertility control in stoats, that rarely achieve normal pregnancies in captive controlled environments. If the analysis shows that developing an abortifacient for stoats is a worthwhile for social reasons, then the next step would be to identify the dose required to abort litters in ferrets, followed by trials in the wild monitoring population trends.

6.2 Recommendations

Based on our study, we make the following recommendations.

- Conduct further experiments with higher/repeated doses of pimozide or prolactin itself on female stoats around March/April. They may still yield a dosing regimen that would confirm or deny the possibility of manipulating this pathway in isolation as a fertility control method.
- If pimozide can induce precocious implantation in stoats at higher doses, conduct additional research to demonstrate that if implantation occurs then any litters are aborted (unlike in mink where pregnancies go to full term and are successful).

- Consider undertaking experiments on the prospects of cabergoline as a fertility control tool (with the dosing during the last 2 weeks of pregnancy). However, the challenges of conducting meaningful experiments of this sort on captive animals means we advise undertaking a cost-benefit analysis of this technique versus progressing toxins such as PAPP.

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