

Stoat reproductive biology

Cheryl O'Connor, Julie Turner, Susie Scobie and Janine Duckworth

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Cover: Stoat (*Mustela erminea*) kits born in captivity. Photo: Grant Morriss, Landcare Research.

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ABSTRACT

Stoats (*Mustela erminea*), which are arguably New Zealand's most destructive predators, are highly fecund, and display precocious sexual maturity and a prolonged period of delayed implantation. However, little is known about their basic biology and reproductive physiology. From July 2000 to December 2003, research on stoat reproductive biology and captive management was undertaken. Initial research focused on the reproductive biology, stress physiology, immunology and behaviour of freshly captured stoats during and following acclimatisation to captivity. Almost all wild-caught stoats had blastocysts present at capture that were present *in utero* for more than 24 weeks after capture. None of the wild-caught females gave birth in captivity. Up to 4 weeks post-capture, immune function was suppressed (depleted lymphocytes and impaired cell proliferative response). Between 6 and 8 weeks post-capture, the proportion of stoats displaying avoidance rather than staying behaviour on contact with humans increased, as did the number of animals accepting food within 1 h of presentation. Consequently, an 8-week period of acclimatisation to captivity is recommended before wild-caught stoats are utilised in physiological research. Visual assessment proved the best technique to assess reproductive activity in naturally bred and manipulated stoats. In females, oestrus was associated with swelling of the vulva and changes in vaginal cytology; in males, reproductive activity was associated with increased testicular size and the presence of sperm in samples. By altering the light-dark cycle, the effect of photoperiod on the control of oestrus, seasonal breeding and the 10-month period of embryonic diapause were investigated. Acute exposure of stoats to simulated summer photoperiods in winter significantly advanced the onset of breeding activity. Light-treated females displayed oestrus 2 months earlier than controls, and treated females that were mated conceived, thus extending the breeding season of captive stoats. The testicular development of males was also advanced. For the first time on record in New Zealand, adult and juvenile females were mated and gave birth to a total of 21 live young in captivity. Nine of the captive-born females are part of the stoat breeding colony that will provide high-quality animals for future stoat research.

Keywords: stoat, *Mustela erminea*, reproductive biology, captive breeding, immunology, acclimatisation, photoperiod

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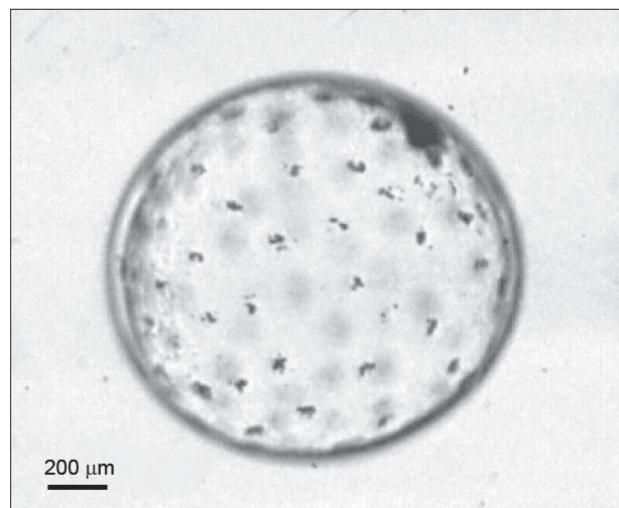
1. Introduction

Stoats (*Mustela erminea*) are one of New Zealand's most destructive predators and are implicated in the continuing decline of native bird species (King 1984; McDonald & Murphy 2000). Biological control has the potential to dramatically increase control effectiveness. For stoats, fertility control may be an appropriate strategy (Barlow & Barron 2005), given the positive progress with the development of such control for mice (*Mus musculus*), rabbits (*Oryctolagus cuniculus*) and foxes in Australia, and possums (*Trichosurus vulpecula*) in New Zealand (Hinds et al. 2000; Norbury 2000). The development of effective fertility control will require a good understanding of stoat reproductive biology, about which little is currently known.

In the wild, females mate between September and November and are pregnant for 8-11 months, with young being born the following September-October. Pregnancy in the stoat is complex. After mating, the fertilised embryo grows until it develops to a fluid-filled ball of approximately 200 cells in size called a 'blastocyst' (Fig. 1). During the next stage of pregnancy, called 'embryonic diapause', the embryo stops growing and remains in the uterus for c. 8-9 months (Amstislavsky & Ternovskaya 2000). This stage of pregnancy is peculiar to stoats; it does not occur in ferrets (*Mustela putorius*) or other mustelids. A consequence of this reproductive strategy is that in the wild more than 90% of stoats are pregnant (in the blastocyst stage) between December and September. In August, the embryo begins to grow again, it implants into the uterine wall ('delayed implantation') and pregnancy continues as in most other mammals to birth (King & Murphy 2005).

To improve our understanding of stoat reproductive biology, the natural stoat reproductive process and its control were investigated from July 2000 to December 2003. We studied the reproductive physiology of captive, acclimatised stoats throughout a natural breeding season, to provide baseline data for experimental treatments. We assessed the reproductive biology, stress physiology, immunology and behaviour of wild-caught stoats during and following acclimatisation to captivity, as initially much of the research depended on wild-caught animals. By altering the light-dark cycle

Figure 1. View of a stoat (*Mustela erminea*) blastocyst (40 × magnification).



(photoperiod) experienced by stoats, we investigated the control of oestrus, seasonal breeding and the prolonged 10-month period of embryonic diapause. We developed techniques to assess and manipulate the reproductive state during oestrus and pregnancy. The knowledge gained was used to optimise husbandry and the welfare of the animals, leading ultimately to the establishment of a captive stoat breeding colony that will benefit research in many areas of stoat control.

2. Objectives

The central theme of this investigation was to improve our understanding of the reproductive biology of stoats in order to provide a platform for future research on fertility control and to enable captive breeding of stoats for research by:

- Analysing existing captive stoat data/samples at the Landcare Research facility for information relevant to breeding success and adaptation to captivity.
- Determining the fate of blastocysts in wild-caught female stoats brought into captivity.
- Determining the period of immunological and behavioural acclimatisation in wild-caught stoats.
- Developing and evaluating techniques to monitor female oestrous activity and male testicular function.
- Developing and testing photoperiod treatments to stimulate out-of-season mating.
- Optimising protocols for the captive breeding of adult and juvenile stoats.

3. Methods

3.1 EXISTING STOAT DATA RELEVANT TO BREEDING SUCCESS

There is very little published data on breeding captive stoats in New Zealand. The limited local and international information has previously been reviewed by McDonald & Lariviere (2002) and King & Murphy (2005).

To obtain historical data from previous Landcare Research projects, 42 dead stoats that had been collected at the Landcare Research Animal Facility, Lincoln, over the previous 4 years were sexed and the reproductive states of the 11 females were assessed for evidence that blastocysts had been retained or reactivated in the captive animals. For comparison, we also studied normal blastocysts and embryos in wild female stoats by examining the reproductive tracts of 11 female stoats that were kill-trapped in the St Arnaud area, Nelson, between July and December 1998.

3.2 ACCLIMATISATION TO CAPTIVITY

All wild-caught stoats (except females in the captive breeding programme—see section 3.5) were housed in individual cages (60 × 150 × 90 cm) under an outside shelter at the Landcare Research Animal Facility, Lincoln. Each stoat was supplied with a nest box (40 × 33 × 15 cm) containing shredded paper as nesting material. Stoats were fed a rotation of dead 1-day-old chicks, chicken pet mince and mutton/beef pet mince in the afternoon, and had free access to water. Captive stoats were assessed for immunological function, behavioural changes and blastocyst survival, as described below.

3.2.1 Immunological tests

Twenty-five captive female stoats were randomly assigned to one of four sampling times at 0, 4, 12 and 24 weeks post-capture (Week 0: $n = 7$; all other times: $n = 6$). At each time point, animals were anaesthetised under fluothane (3–5% fluothane in 2 L O₂/min, delivered by mask) and 2–4 mL of blood was collected by heart puncture. Animals were killed while still under anaesthetic by the intracardial administration of 0.5 mL of pentobarbitone. White blood cells were collected for lymphocyte culture by layering heparinised blood over a ficoll density gradient. The separated cells were washed twice in phosphate buffered saline before being resuspended in RPMI media containing 10% foetal calf serum and antibiotics. Cells were cultured for 72 h with either media or the T cell and B/T cell mitogens Concanavalin A (ConA; 10, 50 and/or 100 µg/mL), Lipopolysaccharide (LPS; 6.25, 12.5 and 25 µg/mL) and Pokeweed mitogen (PWM; 3, 6 and 12 µg/mL). Lymphocyte transformation responses to the mitogens were measured by labelling dividing cells with radioactive thymidine. Thymidine uptake provides a measure of the ability of immune cells involved in the cell-mediated arm of the immune system to proliferate in response to T and B cell stimuli. For continuous variables, differences between treatment groups were assessed by Analysis of Variance (ANOVA). Data were log₁₀ transformed prior to analysis to satisfy the assumptions of ANOVA, and differences between treatment means were determined using Tukey's highly significant difference test.

3.2.2 Behavioural responses

Both 'feeding' and 'behaviour response' tests were undertaken on 31 stoats (including 18 females from the immunological tests and 13 males) weekly. Animals were sourced and housed as described above. We based these acclimatisation tests on those previously undertaken on possums, which measured the behavioural response of possums to a standard presentation of food and established a 4-week acclimatisation period in captivity for wild-caught animals (Day & O'Connor 2000). Since stoats are not visible during routine handling and feeding, once a week we recorded whether the chick had been taken from the food bowl (eaten or cached) 1 h after feeding. Following this, the chick remained available to each animal as per their normal feeding regime, and the food bowl was checked again 24 h later. Our assumption was that acclimatised stoats would recognise the sounds of 'food presentation' and would be relaxed enough to feed within the first hour.

On a different day from the feeding test, the immediate behavioural response of each stoat to the door of its nest box being shut was assessed. One response was recorded for each animal, each week. Our hypothesis was that acclimatised stoats would be increasingly visible (i.e. Look and Movement responses) and would not feel threatened (i.e. Vocalisation) as they became less fearful of humans.

The responses were defined as:

- Vocalisation—usually a ‘bark’
- Look—watch observer
- Movement—movement but still visible
- Hide—movement to hide/nest in paper
- Conceal—movement but stay concealed within paper
- No response—no noise or movement at all

Information from this work was also used to optimise animal husbandry protocols for handling newly captured stoats and to determine how long a wild-captured stoat should be left to acclimatise to captivity before being used for experiments.

3.2.3 Fate of blastocysts

To assess possible reasons for why captive stoats have not successfully bred (i.e. produced live young) in New Zealand, we also monitored losses of blastocysts during acclimatisation of the 25 female stoats to captivity. Groups of females were assessed for blastocyst survival at 0, 4, 12 and 24 weeks after capture to specifically assess how the stress of capture affects blastocyst survival in wild-caught stoats. Animals were randomly assigned to treatment groups as described in section 3.2.1.

Animals were killed under fluothane anaesthetic by the intracardial administration of 0.5 mL of pentobarbitone. After removal of the reproductive tract from each animal and examination for sites of embryo implantation, the tracts were weighed and measured. Following careful dissection of the ovaries from the oviduct bursa, the numbers of follicles and corpora lutea present on the ovaries were recorded. Blastocysts could be seen through the translucent uterine wall of freshly killed stoats using a dissecting microscope (20–100 × magnification) (Fig. 2). The oviduct and uterus were each flushed separately from the anterior end with 1–2 mL of phosphate buffered saline (pH 7.4) and any blastocysts that were present were recovered. For continuous variables, effects were assessed by ANOVA and differences between treatment means were determined using Tukey’s highly significant differences test.