Characterization of oestrus and timed collection of oocytes in the grey short-tailed opossum, *Monodelphis domestica*

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**Summary.** A system of test-pairing was used to detect reproductive behaviour in the grey short-tailed opossum. This enabled timing and characterization of the development of pro-oestrous and oestrous behaviour, and facilitated collection of ovulated and unovulated oocytes. Oestrus was induced 8.5 days (n = 80, 95% confidence limits 7.56–9.21) after the introduction of a male. Timed examination of the ovaries by laparotomy indicated that ovulation occurred 14–16 h after the first onset of oestrous behaviour. The development of follicles was linked to pro-oestrous behaviour, and ovulation occurred in the absence of copulation. Vaginal exfoliative cytology indicated that pro-oestrous behaviour was associated with an increasing number of keratinized epithelial cells, and at the time of maximum receptivity to males, a heavy infiltration of polymorphonuclear leucocytes was seen. Oocytes were typically marsupial: large (approximately 250 μm in diameter), with a yolky vitellus and thin zona pellucida. An average of 6 oocytes were ovulated per ovary.

**Introduction**

The structure of the gametes, events at fertilization and early embryonic development in marsupials are markedly different from those of eutherian mammals (Hartman, 1923; Rodger & Bedford, 1982). Our understanding of the biological significance of some of these differences stems mainly from investigations in which marsupial oocytes are recovered from the genital tract after mating. In a number of species (macropodids, *Didelphis*) removal of pouch young will generate ovarian follicular growth and oestrus after a variable time period (Renfree & Tyndale-Biscoe, 1978). In small marsupials, such as dasyurids, this technique is not effective as extensive behavioural oestrus is exhibited; consequently careful and frequent observations of behaviour are required if oocytes are to be collected after spontaneous ovulation (Selwood, 1982). Predictable timing of oocyte recovery in a breeding laboratory colony of marsupials would provide a valuable tool in the elucidation of metatherian gamete physiology and fertilization.

The grey short-tailed opossum, *Monodelphis domestica*, is a South American didelphid (80–120 g body weight) which reproduces under ordinary laboratory conditions (Fadem et al., 1982). Even in this amenable species generation of ovulation on demand has so far been unobtainable. Reports have shown that the presence of a male activates oestrus as evidenced by behaviour and pregnancy (L. M. Baggott, unpublished observations), and by inference as seen from exfoliative vaginal cytology and pregnancy (Fadem, 1985). However, in isolation, females have been claimed to exhibit oestrous cycles as indicated by exfoliative vaginal cytology ranging from 10 to 32 days in length (Fadem & Rayve, 1985).

In this study behavioural oestrus has been characterized after controlled exposure of females to males. Distinct behavioural cues were temporally correlated with ovulation, allowing oocytes to be collected at various stages of maturation for fertilization and embryological studies.
**Animals and maintenance**

A breeding colony of grey short-tailed opossums, numbering over 250, has been established from an original 39 animals which were obtained from the Southwest Foundation for Biomedical Research, San Antonio, Texas, in 1983. The animals used in this study were from this homebred colony.

The animals were housed in rooms of floor area 3·5 × 3·5 m, with about 75 animals per room. Breeding pairs were caged in polypropylene rat boxes, 56 × 38 × 18 cm, with flat wire tops. Aluminium nesting boxes, 18 × 13 × 10 cm, were also provided. The bedding material consisted of tissue paper, and wood chips covered the cage floors. Single animals were housed in polypropylene stock cages, 44 × 28 × 15 cm, with raised wire tops. Tissue and sawdust were provided for bedding.

A reversed 14 h light:10 h dark photoperiodic cycle with white fluorescent lights was used, with lights off at 11:00 h. The temperature was maintained at 24°C, and humidity at 50%.

The animals were fed once per day during the first part of the dark period with pelleted fox food (Kemovit: Cooper Supply Co., Bristol, U.K.), and water was always available. This basic diet was supplemented in nursing females with 14 g minced beef or freshly killed baby mice twice per week.

**Procedure for the assessment of the development of pro-oestrous and oestrous behaviour**

**Pairing.** Females of minimum age 5 months which had failed to litter, or which had not previously been kept with a male for a minimum of 16 days, were paired with unrelated males of minimum age 5 months. The male was placed inside a wire box, 18 × 13 × 10 cm with 1 cm mesh, which was then put into the female’s cage for 3 h on 3 consecutive days. On the 4th day, the male was placed into the female’s cage in the wire box for 1 h, and was then released into her cage. This was the day of pairing. The animals were checked for aggressive behaviour at regular intervals over the next 24 h. If this occurred, they were separated, and the procedure repeated with a different male. The pair were then left together for 18 days, after which time (a) the male was removed and separately caged, and the female checked daily for offspring; or (b) the female was ‘test paired’ (see below) for behavioural pro-oestrous.

**Test pairing.** Between 3 and 5 h into the dark period, females which had been paired for 5 days were put into a clean stock cage, 56 × 38 × 18 cm, and a strange, unpaired male was immediately introduced to her. The behaviour of both animals was then assessed using the behaviours listed in Table 1. This procedure was repeated daily at the same time until pre-copulatory behaviour was observed. Once this was seen, test pairing was repeated at more frequent intervals until the female was fully receptive. The end of behavioural pro-oestrous was marked by the willingness of the female to enter into a complete sequence of behaviour patterns which culminated in copulation with a strange male. A different unpaired male was used for each test pairing of any one female.

The following variations on this basic procedure were carried out. (1) Castrated males were used for pairing and intact males were used to test the females for behavioural pro-oestrous. (2) Intact males were put into the wire box for 3 h on 10 consecutive days. After 5 days, daily test pairing was carried out (see below). Thus the paired male was not allowed contact with the female other than across the wire of the box, and the test male was removed from the testing box at the first show of aggressive behaviour.

**Estimation of time of oestrous.** The time of onset of behavioural oestrous in relation to initial pairing was calculated by subtracting 14 (the length of pregnancy, Fadem et al., 1982) from the total number of days which the animals were kept together. This observation was made in 79 pairings of 31 breeding females in which litters were produced. The following criteria were fulfilled: (i) the animals were kept together for a minimum of 18 days; (ii) both animals were of proven fertility; (iii) the pairing was non-aggressive; (iv) both animals were intact; (v) the regimen of pairing and separation did not deviate from that outlined above; (vi) both animals were 5–24 months old.

In a follow-up investigation, 36 pairings of 29 females were set up for a maximum of 12 days, and they were then test-paired for behavioural oestrous (see above). This reduced pairing time was used because the former regimen had established that, if oestrous was induced, 85% of all such inductions occurred within 12 days of pairing.

The females used for direct observation of oestrous included those for which more than one observation was made (replicates) and those for which only one observation was made (singles). The results from these two groups were compared using the Mann–Whitney (non-parametric) test to establish that these groups were derived from the same distribution.

For animals for which oestrous was determined from parturition date and those for which behavioural oestrous was observed directly, the median time (+95% confidence limits) of the appearance of oestrous after pairing was calculated from only those animals in which oestrous was induced.

**Surgical investigation of the female tract in relation to reproductive behaviour**

**Laparotomy.** Seventeen females were anaesthetized with ether before surgical examination of the reproductive tract. An area of skin about 2 cm² on the animal’s flank was shaved, and a lateral incision made parallel to the crest of the pelvis. The oviduct and ovary were found lying just below the musculature. Using an operating microscope, assessment was made of follicle development on the ovary, presence of recent ovulation sites or corpora lutea, and size of the uterus.
whether separated containing and drop oviduct was on Collection no Histological BWW transferred Herts, during After the Investigation pro-oestrous By By Ovaries All then were severed distal oviducal flushing. By Ham's medium oocytes from pro-oestrous (Biggers et al., 1971) or Dulbecco's modified Eagles medium (Gibco Ltd, Paisley, U.K.). The oviduct was then severed at the utero-tubal junction, and a needle inserted into the open end. Medium was flushed through the oviduct from a 1-ml syringe attached to the needle. This was repeated twice. The oocytes were then pipetted into a fresh drop of Ham's F10 medium under oil.

All oocytes were incubated in 5% CO₂ at 37°C unless immediately fixed for histology.

Histological assessment of events in the female reproductive tract associated with behavioural oestrous

Ovaries were removed from animals during anoestrus (i.e. after separate housing for 3 months, during which time no pro-oestrous or oestrous behaviour was seen), just before ovulation (i.e. immediately after the first pairing of an oestrous period) or after ovulation (confirmed by laparotomy).

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### Table 1. Test-pairing behaviour score sheet for grey short-tailed opossums

<table>
<thead>
<tr>
<th></th>
<th>Initiated by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td><strong>Anoestrus</strong></td>
<td></td>
</tr>
<tr>
<td>1. Antagonistic behaviour</td>
<td></td>
</tr>
<tr>
<td>1.1 Open mouth threat</td>
<td>+</td>
</tr>
<tr>
<td>1.2 Attack leap</td>
<td>+</td>
</tr>
<tr>
<td>1.3 Biting</td>
<td>+</td>
</tr>
<tr>
<td>1.4 Locked fighting</td>
<td>+  +</td>
</tr>
<tr>
<td>1.5 Forcible genital sniffing</td>
<td>+</td>
</tr>
<tr>
<td>1.6 Squeaking</td>
<td>+</td>
</tr>
<tr>
<td><strong>Pro-oestrus</strong></td>
<td></td>
</tr>
<tr>
<td>2. Pre-copulatory behaviour</td>
<td></td>
</tr>
<tr>
<td>2.1 Naso-cloacal contact</td>
<td>+  +</td>
</tr>
<tr>
<td>2.2 Slow circling</td>
<td>+  +</td>
</tr>
<tr>
<td>2.3 Chasing</td>
<td>+</td>
</tr>
<tr>
<td>2.4 Rump dragging</td>
<td>+</td>
</tr>
<tr>
<td>*2.5 Chattering</td>
<td>+  +</td>
</tr>
<tr>
<td>2.6 Kneading</td>
<td>+</td>
</tr>
<tr>
<td><strong>Oestrus</strong></td>
<td></td>
</tr>
<tr>
<td>3. Copulatory behaviour</td>
<td></td>
</tr>
<tr>
<td>3.1 Mounting</td>
<td>+</td>
</tr>
<tr>
<td>3.2 Abdomen grasping</td>
<td>+</td>
</tr>
<tr>
<td>3.3 Ankle grasping</td>
<td>+</td>
</tr>
<tr>
<td>3.4 Lying on side</td>
<td>+  +</td>
</tr>
<tr>
<td>3.5 Pelvic thrusting</td>
<td>+</td>
</tr>
<tr>
<td>3.6 Intromission</td>
<td>+</td>
</tr>
<tr>
<td>3.7 Locking</td>
<td>+  +</td>
</tr>
</tbody>
</table>

*Transition period.

After closing the incision with suture, the animals were kept warm and under observation. Some salivation occurred during anaesthesia, but recovery from the anaesthesia was within 10 min, and apparently trouble-free.

**Investigation of nature of ovulation.** Three of the females were paired and test-paired as outlined above, but were separated at the first indication of pro-oestrus. Laparotomies were then performed at about 8-h intervals to determine whether ovulation occurred in the absence of mating. Great care was taken during the surgery not to stimulate the cervices or uteri of females.

**Collection of oocytes.**

*By ovariection.* Ovaries were removed and placed in Ham's F10 medium (Flow Laboratories, Rickmansworth, Herts, U.K.). Follicles were then carefully punctured with a fine needle using a dissecting microscope. The oocytes were transferred to 200 µl drop of fresh medium under silicone oil.

*By in-vivo follicle puncture.* During laparotomy, follicles were carefully punctured using a fine glass pipette containing Ham's F10 medium. The oocytes were drawn into the pipette using a 0-1 ml syringe attached to a cannula and were then transferred to fresh medium as above.

*By oviducal flushing.* The female was hemi-hysterectomized by tying off one uterus, and cutting through the uterus on the distal side of the ligature. The ovary, oviduct and uterus from that side were removed to a Petri dish containing BWW medium (Biggers et al., 1971) or Dulbecco's modified Eagles medium (Gibco Ltd, Paisley, U.K.). The oviduct was then severed at the utero-tubal junction, and a needle inserted into the open end. Medium was flushed through the oviduct from a 1-ml syringe attached to the needle. This was repeated twice. The oocytes were then pipetted into a fresh drop of Ham's F10 medium under oil.

All oocytes were incubated in 5% CO₂ at 37°C unless immediately fixed for histology.
Ovaries were fixed in 2% glutaraldehyde, dehydrated, paraffin wax embedded, sectioned and stained with haematoxylin and eosin.
Oocytes were examined with phase-contrast micrography, and photographed.
About 0.5 ml of sterile normal saline (9 g NaCl/l) was introduced into the urogenital sinus of a hand-held female via a smooth-ended pipette. The saline was withdrawn and placed on a clean glass slide. It was then observed with phase-contrast microscopy, scored for cell types and photographic records were made.

Results

Development of pro-oestrous and oestrous behaviour

A non-receptive female displayed antagonistic behaviour towards the strange male used for testing receptivity. When on subsequent testing she showed amicable behaviour, as characterized by acquiescence to genital sniffing (by the male), the female was considered to have exhibited the first signs of behavioural pro-oestrus. Nine observations of the total period of pro-oestrus showed a range from less than 24 h to a maximum of 72 h. As pro-oestrus developed, the range of behaviour patterns increased in the sequence shown in Fig. 1.

The sequence of behaviour leading up to copulation as pro-oestrus developed seemed to be initiated by the female. Until she was properly receptive, her behaviour appeared to invite the male but prevent him from making further courtship advances. Once a female had progressed more than 10 h into the period of behavioural pro-oestrus, she behaved in the same way towards each strange male introduced during any single testing session. Behavioural oestrus was assumed to have begun when a female was willing to accept copulatory behaviour leading to mating (see Table 1).

Some pro-oestrous behaviour (i.e. repeated chasing and retreating, hunching) was noted once out of 6 times when females were paired with castrated males; however, this female did not enter behavioural oestrus with any of the test males used. Of 6 females which were paired with males kept

![Fig. 1. Record of development of pro-oestrus in a single animal as assessed by timing of precopulatory behaviour. Black dots represent observations, and hatched blocks represent dark period.](image-url)
in the wire box and not allowed any period of free interaction with the female, none showed behavioural pro-oestrus within 14 days of this pairing procedure.

Anoestrus was characterized by female aggression towards strange males. Under test-pairing conditions, males have never been seen to attack females, although it has been noted during the development of the colony that deaths due to fighting have always been of the female of a heterosexual pair. Aggression by females during test pairing was characterized by open-mouthed threats and, occasionally, locked fights occurred between test pairs of a strange male and an anoestrous female.

The median time between pairing and onset of behavioural oestrus as derived from date of parturition was 8.5 days ($n = 80, 95\%$ confidence limits 7.56–9.21 days). The frequency distribution can be seen in Fig. 2. In those pairings in which behavioural oestrus was observed directly by daily test pairing, females ($n = 37$), paired only once or a number of times, did not differ significantly in the median time of oestrus after test pairing. The median time between pairing and oestrus in the combined single and replicate groups was 8.6 days, with 95% confidence limits of 7.6–9.6 days. Of paired females, 13-9% showed no behavioural oestrus as judged by non-appearance of litters within 28 days of the pairing.

*Follicular development and ovulation*

Matings occurred over a 16–18-h range, although apparent receptivity, which included a period of pro-oestrus, exceeded 18 h, sometimes by as much as 66 h. The results of the laparotomies (Table 2) suggested that follicular development progresses over the first 14–16 h of behavioural oestrus and that ovulation occurs at that time. In no case was ovulation detected before the onset of full behavioural receptivity, or mating observed after ovulation had been determined by laparotomy.

In the 3 females which were separated at the first indication of pro-oestrus, ovulation was seen by laparotomy to have occurred 14–16 h after the onset of behavioural oestrus, although copulatory behaviour had been prevented by removal of the male.

Unovulated oocytes collected within 2 h of the beginning of behavioural oestrus by follicle puncture measured 250 μm in diameter (see Fig. 4), and one ovary yielded ~6 preovulatory oocytes.
Table 2. Summary of observations on ovaries and oocytes made during the period of behavioural oestrus

<table>
<thead>
<tr>
<th>Period from beginning of behavioural oestrus (h)</th>
<th>No. of animals</th>
<th>Ovaries</th>
<th>Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>5</td>
<td>Large follicles</td>
<td>Pre-ovulatory, no polar bodies</td>
</tr>
<tr>
<td>2–7</td>
<td>6</td>
<td>Large follicles</td>
<td>Pre-ovulatory, first body extruded, telophase I</td>
</tr>
<tr>
<td>14–18</td>
<td>3</td>
<td>Ovulation sites plus large follicles</td>
<td>Pre-ovulatory with first polar body, or ovulated with no inner tertiary egg membrane</td>
</tr>
<tr>
<td>16–22</td>
<td>8</td>
<td>Ovulation sites, corpora lutea</td>
<td>Oocytes in oviduct; ± first deposition of inner tertiary egg membrane</td>
</tr>
</tbody>
</table>

These oocytes had some follicle cells adhering, but it was possible to dislodge these by gentle pipetting. Oocytes collected up to 1 h after the onset of behavioural oestrus appeared to have no polar bodies but in oocytes which were collected 2 h after oestrus was first observed, the first polar body had been extruded (see Fig. 4). Tubal flushings also yielded an average of 6 oocytes from each oviduct. Oocytes flushed from the oviduct 17 h after first pairing (beginning of behavioural oestrus) had zonae pellucidae, but no mucin deposition. These oocytes appeared to be similar to those obtained by follicle puncture. The flushed oocytes were devoid of follicle cells and may be presumed to be fertilized. Oocytes obtained by flushing 22 h after the onset of behavioural oestrus had a thin mucin layer laid down (see Table 2).

The histological changes which occurred in the ovary were similar to those seen in *Didelphis virginiana* (Hartman, 1923; Martínez-Esteve, 1942), and thus follow the familiar mammalian pattern. During anoestrus, the ovary contained many small primary follicles and atretic follicles. Few remains of corpora lutea were seen. The development of follicles on the ovaries was associated with pro-oestrous behaviour, and at behavioural oestrus large follicles could be seen on the surface of pre-ovulatory ovaries (Fig. 3). After ovulation, during the 10–20 h after the oestrous period, large corpora lutea were present. The number of these seen in sections of post-ovulatory ovaries corresponded with the number of oocytes recovered.

**Vaginal cytology**

During anoestrus, any epithelial cells were small and nucleated. Over the course of pro-oestrous, the proportion of keratinized to non-keratinized cells increased until nearly all were of the former type.

**Fig. 3.** Preovulatory ovary showing two secondary follicles. H & E, × 20.

**Fig. 4.** Unovulated oocyte recovered 5 h after first observed mating, showing presence of the first polar body (pb). Unstained, phase-contrast, × 600.

**Fig. 5.** Pro-oestrous vaginal cytology. The cells become increasingly cornified, and as pro-oestrous progresses more non-nucleated cells are seen. Unstained, phase-contrast, × 150.

**Fig. 6.** Oestrous vaginal cytology. Polymorphonuclear leucocytes (▼) are present in large numbers at the time of maximum behavioural receptivity. Unstained, phase-contrast, × 150.
type. There were also many more cells present at this time (see Fig. 5). At the time of maximum receptivity, small polymorphonuclear leucocytes became present in increasing numbers. There was then a decline in the number of epithelial cells until the vast majority of cells present were leucocytes (see Fig. 6). Vaginal smears taken immediately after mating rarely revealed spermatozoa as the insemination took place higher in the tract than the position from which the smear was obtained. During the post-oestrous period, the smears returned gradually to an essentially acellular state.

**Discussion**

Although little is known of the natural ecology of these opossums, there is no reason to suppose that the development of pro-oestrous behaviour as observed in this study would be different from that which would present in the wild. This pattern of behaviour may have the adaptive advantage of maintaining the male's interest until full oestrus occurs. Since grey short-tailed opossums appear to ovulate in the absence of copulatory stimulation, the development of defined pro-oestrous behaviour would be of prime importance in synchronizing copulation with ovulation, if the chance of successful fertilization is to be maximized. This is in contrast to such species as dasyurids (Selwood, 1982) in which mating (followed by the storage of spermatozoa in the female tract) may precede ovulation by more than 1 week. How long Monodelphis spermatozoa remain viable in the female tract is unclear although the ejaculate of American marsupials contains far fewer spermatozoa than those of the comparable Australian species (Bedford et al., 1984).

The observed range of pro-oestrous length, less than 24 h to a maximum of 72 h, was derived from different animals, and so no conclusion can be drawn about whether the length of pro-oestrus was the characteristic of the pair of individual animals, or varied from cycle to cycle. It is clear, however, that the development of pro-oestrous behaviour seemed to depend upon prolonged free interaction with an intact male. Our preliminary findings indicate that physical as well as olfactory contact is necessary. The failure of the castrated males to elicit pro-oestrous behaviour in females with which they were paired supports the argument put forward by Fadem (1985) that reproductive hormones are in part responsible for the role played by the male in initiating the development of pro-oestrous behaviour in the female opossum. In the absence of a male, females did not cycle spontaneously. The results of Fadem & Rayve (1985) suggest a bimodal distribution of oestrous cycle length of 14-4 days and 32-3 days and that females housed alone were less likely to show oestrous cycles. In our experience, females did not show any regular cyclicity in oestrous behaviour. Firstly, females in which oestrus was induced but conception was not allowed failed to return to oestrus unless paired with a male, and secondly, the majority of females came into oestrus 6-10 days after pairing with a male. With females showing regular oestrous cycles a broader range would be expected. The 13.9% of failures to litter (and by inference come into oestrus) suggests that the females may have a refractory period after the previous ovulation or parturition, during which induction of oestrus by the male may be impossible.

The 18-h duration of behavioural oestrus in Monodelphis is in contrast to that for two other didelphids which have been studied: up to 3 days in Marmosa (Godfrey, 1975) and 36 h in Didelphis (Reynolds, 1952). Trupin & Fadem (1982) found receptivity in Monodelphis to last for 36 h; however, the extended period of pro-oestrus seen in some females may account for this discrepancy. Rodger & Bedford (1982) found Didelphis to ovulate 12-15 h after the beginning of behavioural oestrus, which is comparable with the 14-16-h period seen in Monodelphis in this study. The evidence of the laparotomies leads to the conclusion that Monodelphis will ovulate, once pro-oestrus is established, without the vaginal or cervical stimulation of copulation. However, it is possible that pre-copulatory behaviour itself stimulates the LH surge which results in ovulation.

The oocytes recovered from Monodelphis (see Fig. 6) resembled the cleidoic vertebrate egg described by Hughes (1977), who considered the oocytes of Trichosurus vulpecula (the brush-tailed possum) as typifying those of marsupials. The presence of the first polar body, as seen in some
epithelial oocytes, indicates that resumption of meiosis during the final maturation of the follicle is rapid, as in *Didelphis* (Hartman, 1916). The deposition of the inner tertiary egg membrane (mucin coat) begins about 20 h after the beginning of oestrus, as estimated by timed recovery of oocytes (L. M. Baggott, unpublished observation). By deduction, therefore, this process starts around 2 h after ovulation. This leaves a narrow window for fertilization, as penetration by spermatozoa is improbable once mucin deposition starts; indeed, they appear *in vitro* to be unable to bind to the egg surface at all (L. M. Baggott & H. D. M. Moore, unpublished data). Rodger & Bedford (1982) have suggested that fertilization rapidly follows ovulation in *Didelphis* and it is possible that early deposition of mucin on to the zona pellucida may constitute a block to polyspermy in the opossum.

Unlike *Didelphis* from which up to 40 oocytes from a post-partum ovulation have been collected (Rodger & Bedford, 1982), the number of ovulations per cycle in *Monodelphis* (up to 14) does not greatly exceed teat number. The greatest rate of loss of oocytes in *Monodelphis* appears to be at the preovulatory stage as a result of atresia. The large bulging follicles seen at the onset of behavioural oestrus in *Monodelphis* are similar in size to those of *Didelphis*, but fewer in number.

As with the ovarian histology, the vaginal cytology of the mature *Monodelphis* is similar to that of *Didelphis*, and indeed most eutherians (Hartman, 1923; Fadem & Rayve, 1985). Although the appearance of polymorphs in the vaginal smear indicates behavioural oestrus, it is not a method that could reliably be used for the purpose of collecting unovulated oocytes. It is possible to correlate the behavioural observations with the cytological changes seen in the smears, but because they were obtained from the urogenital sinus rather than from the lateral vaginae, in which the cyclic epithelial changes occur, the actual timing of reproductive events cannot accurately be monitored.

In summary, this study has characterized a reproducible technique for inducing ovulation in *Monodelphis domestica*, and for the collection of oocytes. We have now used this procedure for fertilization and early embryonic studies in this species.

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