

## Ovarian activity reversibility after the use of deslorelin acetate as a short-term contraceptive in domestic queens

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Received 8 October 2011; received in revised form 20 March 2012; accepted 20 March 2012

### Abstract

The objective was to evaluate ovarian activity reversibility in domestic queens after short-term contraceptive treatment with deslorelin acetate. Ten mature queens were used. In all queens, the estrous cycle was evaluated every 72 h by vaginal cytology (VC) and behavior assessments. When queens had VC characteristic of interestrus or diestrus, one deslorelin acetate implant (4.7 mg) was placed in the subcutaneous tissue of the interscapular region (day of insertion = Day 0). Thereafter, VC was performed every 48 h and on Day 90, implants were removed. At Day 100, estrus and ovulation were induced with 100 IU eCG (im), followed by 100 IU hCG (im), 84 h later (Day 103.5). Queens were ovariectomized on Day 106. Corpora lutea (CL) were counted, oviducts were flushed, and oocytes were identified, isolated and stained to assess viability. In all queens, blood samples for plasma progesterone concentrations were collected once a week, from Days –21 to 106. After deslorelin acetate application, four queens had VC and behavior typical of estrus, and one ovulated. Furthermore, ovulation occurred in three queens that did not have VC or behavior consistent with estrus. After the initial ovarian stimulation, all females had anestrous VC during the deslorelin treatment period. Implants were readily removed. Following implant removal, all females responded to treatments to induce estrus and ovulation. There were (mean  $\pm$  SEM) 13.1  $\pm$  5.5 CL and 8.1  $\pm$  5.5 oocytes per queen; the oocyte recovery rate was 56.8  $\pm$  25.4% and all recovered oocytes were viable. We concluded that deslorelin acetate can be used as a reversible short-term contraceptive in domestic cats, because estrus and ovulation were successfully induced following implant removal. © 2012 Elsevier Inc. All rights reserved.

**Keywords:** Deslorelin acetate; Contraception; Domestic cat; Oocyte recovery

### 1. Introduction

Gonadotrophin releasing hormone (GnRH) is a decapeptide that belongs to a group of neuropeptides of hypothalamic origin which control secretions of the anterior pituitary. It is well known that GnRH influ-

ences reproductive processes, mainly by regulating pituitary gonadotropin synthesis and release, which in turn modulate steroidogenesis and gametogenesis [1].

Continuous exposure to GnRH reduces its secretion by downregulating GnRH receptors; this has been used for reversible contraception [2]. In females, exogenous GnRH agonists initially causing a gonadotropin release, which enhances folliculogenesis and may cause ovulation, followed by downregulation and ovarian quies-

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cence [3]. Only three GnRH agonists have been reported as contraceptive in female felids: leuprolide [4,5], deslorelin acetate [3] and azagly-naferalin [6].

Munson, et al. [3] reported for the first time the use of deslorelin acetate (6 mg per animal) as contraceptive in domestic queens. In that study, treated females remained in anestrus for up to 14 mo. Although GnRH agonists have been described as a reversible contraceptive, few studies have specifically demonstrated reversibility. Returns to estrus following the use of deslorelin acetate have been considered asynchronous [3]. Ackermann, et al. [7] treated queens for 60 days with deslorelin acetate (4.7 mg) and reported that they return to normal cyclicity, on average, 42 days after implant removal.

In clouded leopard (*Neofelis nebulosa*) females, only one of seven animals ovulated and developed a normal luteal phase after two injections of leuprolide acetate (3.75 mg/animal) were given for contraception [5]. The authors considered this response inconsistent and attributed it to a severe ovarian alteration and desensitization to hormonal stimulation. This was not expected, because clouded leopards are known for their hypersensitivity to exogenous gonadotropins.

Inconsistent responses were not restricted to feline females [3,8]; similar results have already been described in other species, including dogs [9,10] and giraffes (*Giraffa camelopardalis*) [11]. When treated with GnRH agonists, common brushtail possum (*Trichosurus vulpecula*) had an estrous cycle and pregnancy, with variations among individual animals in the interval needed to restore cyclicity [12].

The first estrus and ovulation induction after 60 days of treatment with deslorelin acetate (4.7 mg/animal) in domestic cats was reported recently [7]. In that study, no ovarian desensitization to hormonal stimulation occurred, despite the efficacy of the contraceptive treatment. Thus, further studies assessing ovarian activity return and ovulation after deslorelin acetate contraceptive treatment should be conducted.

The aim of this study was to evaluate ovarian activity after short-term contraceptive treatment with deslorelin acetate in queens.

## 2. Materials and methods

### 2.1. Animals

Ten mature mixed-breed queens (2–3 yrs old) were kept in an experimental cattery at the Department of Veterinary Surgery and Anesthesiology, Faculty of Veterinary Medicine and Animal Science, FMVZ,

Unesp, Botucatu, SP, Brazil. There was 12 h light/day, with at least 150-lux intensity. The animals were fed commercial cat food (Royal canine Queen 34 Royal canine, Descalvado, SP, Brazil) and water *ad libitum*. Before the study began, all cats were vaccinated against common feline viruses (feline herpesvirus, feline calicivirus, feline panleukopenia) and rabies. Ultrasonography was used for the general examination of reproductive tracts.

### 2.2. Estrus cycle monitoring

In all females, the estrous cycle was evaluated by vaginal cytology (VC) and behavior observations, conducted every 72 h by one trained observer, at 0900 to 1000. Estrous signs were detected by manual stimulation of the hindquarters for 5 to 10 min. For the procedure, each queen was individually placed on a table and hindquarters were stimulated manually. The signs observed were tail deflection, spinal flexion, rubbing or rolling, vaginal discharge, vocalization, treading of the hind legs, body or tail tremor and rigidity, and any striking out, clawing and scratching, or obvious discomfort on manipulation (grunting and escape attempt).

For VC, females were physically restrained and the vulva region was cleaned with dry gauze. Then, a gynecologic brush was introduced cranially in the vaginal canal and delicately rotated. The material collected was transferred to a glass slide, stained with rapid panotic (Panotico Rápido LB Laborclin Produtos Para Laboratórios, Ltda, Pinhais, PR, Brazil) and evaluated under light microscopy (200 or 400 x). One hundred cells were classified according to criteria proposed by Johnston, et al. [13].

### 2.3. Short-term contraceptive treatment

When queens had VC characteristic of interestrus or diestrus (Day 0) evidenced by  $\leq 70\%$  superficial epithelial cells, the queens were anesthetized with ketamine hydrochloride (15 mg/kg im; Dopalen, Vetbrands do Brasil, Paulínia, SP, Brazil) and xylazine hydrochloride (1 mg/kg im; Xilazin, Syntec do Brasil, Cotia, SP, Brazil). A deslorelin acetate implant (Suprelorin, Peptech Animal Health Pty, Limited, NSW, Australia; 4.7 mg/animal) was inserted in the subcutaneous tissue of the interscapular region (the area had been shaved and cleaned). After implant insertion, VC was performed every 48 h. On Day 90, the females were again anesthetized and the implants were removed through an incision on the interscapular region.

#### 2.4. Estrus and ovulation induction

On Day 100, queens received a single injection of eCG (100 IU im; Novormon, Bioniche, Belleville, ON, Canada) to induce estrus and 84 h later (Day 103.5), an injection of hCG (100 IU im; Vetecor, Laboratorios Calier, Spain) to induce ovulation [14].

#### 2.5. Oocyte recovery and viability

At Day 106 the females were ovariectomized. The oviducts were flushed with Dulbecco phosphate buffered saline (DPBS; Nutricell, São Paulo, SP, Brazil) at 38 °C and the fluid recovered was observed under a stereomicroscope. Oocytes were isolated and stained with bisbenzimidazole (Hoechst 33342) and propidium iodide (Sigma-Aldrich, Seelze, Germany) and viability assessed with fluorescent microscopy (Leica DM LB - blue Filter 535 and 617 nm); oocytes with blue fluorescence were considered viable. Corpora lutea (CL) were counted and the oocyte recovery rate was calculated: number of oocytes identified/number of CL x 100.

#### 2.6. Blood collection and hormonal assays

In all queens, blood collection was done once a week, from Days -21 to 106. Blood was centrifuged (3000g for 15 min) and plasma was transferred to 1.5 mL Eppendorf tubes and stored at -20 °C pending analysis. Plasma progesterone (P4) concentrations were measured by a solid-phase RIA kit (Coat-A-Count kit, Diagnostics Products Corporation, Los Angeles, CA, USA), validated for domestic cats [15]. Ovulations were confirmed when plasma P4 concentrations exceeded 1 ng/mL. The intraassay variation coefficient was 6.84% and sensitivity was 0.0001 ng/mL.

#### 2.7. Statistical analyses

No formal analyses were done. Data were shown as mean  $\pm$  SEM, except plasma P4 concentrations (mean  $\pm$  SD).

### 3. Results

#### 3.1. Short-term contraceptive treatment

At the time of implant insertion, five queens were in diestrus, whereas the other five were in interestrus; therefore, half of the queens had spontaneous ovulation. After deslorelin acetate application, no reaction at the application site was observed. Insertion of the implant under anesthesia ensured safe and accurate placement with minimal discomfort. One queen subsequently de-

veloped a mild pyodermatitis at the insertion site because of scratching.

Four females had VC and typical estrus behavior starting  $3.8 \pm 2.2$  days after implant application, with clinical signs of estrus persisting for  $3.5 \pm 3.1$  days. Only one of these females subsequently had increased plasma P4 concentrations, indicating that ovulation had occurred after implant application. Three other queens had increased plasma P4 concentrations, but did not have VC consistent with estrus or typical estrus behavior. Overall, 40% females ovulated after deslorelin acetate short-term contraceptive treatment. The luteal phase lasted at least 5 wk in all queens that ovulated, before or after deslorelin treatment.

After initial ovarian stimulation, there were no additional signs of estrus or ovulations; all females had anestrus VC for the remainder of the short-term contraceptive treatment period. Plasma P4 concentrations from Days -21 to 106 are shown (Fig. 1).

#### 3.2. Implant removal and induction of estrus and ovulation

Implants were readily identified and removed without fragmentation, with minimal scar tissue formation. All queens responded positively to the estrus and ovulation induction protocol, with estrous VC (>70% superficial epithelial cells), typical estrus behavior (tail deflection, spinal flexion, rubbing or rolling, vaginal discharge, vocalization, treading of the hind legs, body or tail tremor and rigidity) and ovulation (Fig. 1) following treatment with exogenous gonadotropins.

#### 3.3. Oocyte recovery and viability

On average, each queen had  $13.1 \pm 5.5$  CL and  $8.1 \pm 5.5$  oocytes. In one female, in addition to the oocytes, two two-cell embryos and one four-cell embryo (Fig. 2) were also recovered, although this female had no direct contact with tom cats. The mean oocyte recovery rate was  $56.8 \pm 25.4\%$  and all oocytes were viable.

### 4. Discussion

In the present study, deslorelin acetate effectively suppressed cyclicity in the domestic cat. Furthermore, after 90 days of treatment, ovarian activity was re-established, with induction of estrus and ovulation and recovery of viable oocytes.

Although domestic cats are known to be induced ovulators, spontaneous ovulation has been reported [16]. Young queens that live in groups [17] and vaginal

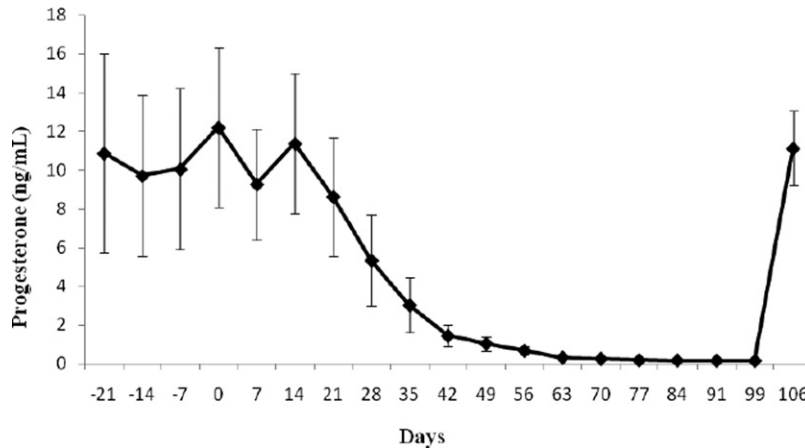


Fig. 1. Mean and SD plasma progesterone concentrations in domestic queens ( $n = 10$  for all data points). Each queen had a deslorelin implant (Days 0–90), was given 100 IU eCG on Day 100, followed by 100 IU hCG on Day 103.5, and ovariectomy on Day 106.

cytology can increase the spontaneous ovulation rate [18]; we inferred that these factors were responsible for the high rate (50%) of spontaneous ovulation before short-term contraceptive treatment.

Initial ovarian stimulation, resulting in estrous VC and typical estrous behavior in four queens, was expected, since it was previously reported in various feline species [8,19]. Munson, et al. [3] reported increased estradiol

concentrations resulting from ovarian stimulation soon after treatment with deslorelin acetate. In the present study, characteristic estrous VC and behavior were observed only in four of 10 queens, but only one of these cats ovulated. Paradoxically, three of the remaining six queens without signs of estrus also ovulated. It is noteworthy that high plasma estrogen concentrations in cats are not consistently accompanied by estrous behavior [20].

Two queens with signs of estrus that did not ovulate were in diestrus at implant insertion; we inferred that this accounted for their failure to ovulate after deslorelin acetate treatment. Ovarian follicular growth during diestrus has been reported in queens [21]. Although luteal failure was observed in bitches treated with deslorelin acetate [22], treatment with deslorelin acetate did not shorten the luteal phase in queens in the present study. However more studies are needed to elucidate whether luteal failure during pregnancy may occur in domestic queens after contraceptive treatment with GnRH agonists.

All queens remained in ovarian quiescence after 20 days from implant insertion, indicating that deslorelin acetate successfully suppressed the ovarian activity and can be used as a short-term contraceptive in domestic cats. This result was similar to previous reports [3,19].

In the present study, removal of the deslorelin acetate implants, followed by exogenous gonadotropins, resulted in ovulation in all queens. This protocol caused superovulation, with more CL than previously described for domestic queens [23]. This response was attributed to the temporary suppression of reproductive activity, because a quiescent ovary stimulated by exogenous gonadotropins is more likely to respond with a predictable number of ovulations [5].

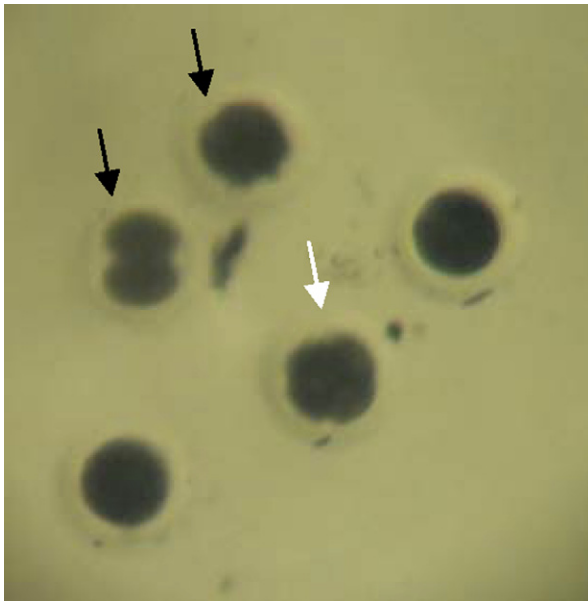


Fig. 2. Oocytes, two-cell embryos (black arrow) and a four-cell embryo (white arrow) recovered from one queen previously treated with deslorelin acetate and subsequently given eCG and hCG to induce estrus and ovulation.



Since 2001, some authors have been using GnRH agonists as a reversible contraceptive. However, few studies have demonstrated reversibility. Severe ovarian desensitization to eCG/hCG treatment in clouded leopards treated with leuprolide acetate for a shorter interval has been described [5]. This was particularly noteworthy, because the leuprolide acetate is less potent than the deslorelin acetate and the clouded leopard is hypersensitive to eCG/hCG treatment.

Recovery of embryos was observed in one female, although there was no contact with tom cats. Therefore, we inferred that this was due to spontaneous oocyte activation. In that regard, parthenogenesis in cats has been described, with a rate varying from 2 to 35.5% [24,25].

This is apparently the first report of oocyte recovery and viability after short-term contraceptive treatment with deslorelin acetate in domestic cats. Further studies should be conducted to determine if treatment duration affects oocyte viability and recovery rate, as well as the embryonic potential development of these oocytes.

In conclusion, deslorelin acetate can be used as a reversible short-term contraceptive in domestic cats, because it was possible to successfully induce estrus and ovulation in this species.

## Acknowledgments

We thank Eunice Oba for conducting hormonal assays and FAPESP for providing financial support. We also thank Peptech Animal Health Pty for the deslorelin acetate implants donation.

## References

- [1] Schneider F, Tomek W, Gründker C. Gonadotropin-releasing hormone (GnRH) and its natural analogues: a review. *Theriogenology* 2006;66:691–709.
- [2] Kutzler M, Wood A. Non-surgical methods of contraception and sterilization. *Theriogenology* 2006;66:514–25.
- [3] Munson L, Bauman JE, Asa CS, Jöchle W, Trigg TE. Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. *J Reprod Fertil Suppl* 2001;57:269–73.
- [4] Pelican KM, Wildt DE, Howard JG. Short-term inhibition of ovarian activity using the GnRH agonist, leuprolide acetate, in the clouded leopard. Fifth International Symposium on Canine and Feline Reproduction, Embu das Artes, São Paulo, Brazil, 2004, p.127–8.
- [5] Pelican KM, Wildt DE, Howard JG. GnRH agonist Lupron (leuprolide acetate) pre-treatments prevent ovulation in response to gonadotropin stimulation in the clouded leopard (*Neofelis nebulosa*). *Theriogenology* 2006;66:1768–77.
- [6] Rubion S, Driancourt MA. Controlled delivery of a GnRH agonist by a Silastic implant (Gonazon) results in long-term contraception in queens. *Reprod Domest Anim* 2009;2:79–82.
- [7] Ackermann CL, Destro FC, Volpato R, Trevisol E, Sousa NR, Derussi AAP, et al. Indução do estro e ovulação em gatas domésticas após o tratamento contraceptivo com acetato de deslorelina (Suprelorin). [Induction of estrus and ovulation in domestic cats following contraceptive treatment with deslorelin acetate (Suprelorin)]. XIX Congresso Brasileiro de Reprodução Animal, Recife, 2011, p. 262.
- [8] Bertschinger HJ, Trigg TE, Fugate AN, Nothling JO, Mabv G. Contraception of lionesses, cheetahs and wild dogs using the GnRH agonist implant Suprelorin®. 6th international Symposium on Canine and Feline Reproduction & 6th biannual EVSSAR Congress, Vienna, Austria, 2008, p. 41–3.
- [9] Wright PJ, Verstegen JP, Onclin K, Jöchle W, Armour AF, Martin GB, et al. Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progesterin. *J Reprod Fertil Suppl* 2001;57:263–8.
- [10] Romagnoli S, Stelletta C, Milani C, Gelli D, Falomo ME, Mollo A. Clinical use of deslorelin for the control of reproduction in the bitch. *Reprod Domest Anim Suppl* 2009;44:36–9.
- [11] Patton ML, Bashaw MJ, del Castillo SM, Jöchle W, Lamberski N, Rieches R, et al. Long-term suppression of fertility in female giraffe using the GnRH agonist deslorelin as a long-acting implant. *Theriogenology* 2006;66:431–8.
- [12] Eymann J, Herbert CA, Thomson BP, Trigg TE, Cooper DW, Eckery DC. Effects of deslorelin implants on reproduction in the common brushtail possum (*Trichosurus vulpecula*). *Reprod Fertil Dev* 2007;19:899–909.
- [13] Johnston SD, Kutritz MVR, Olson PNS Canine and Feline *Theriogenology*. Saunders, 2001, p. 591.
- [14] Villaverde AI, Melo CM, Martin I, Ferreira TH, Papa FO, Taconeli CA, et al. Comparison of efficiency between two artificial insemination methods using frozen-thawed semen in domestic cat (*Felis catus*): artificial insemination in domestic cats. *Anim Reprod Sci* 2009;114:434–42.
- [15] Swanson WF, Graham K, Horohov DW, Thompson DL Jr, Godke RA. Ancillary follicle and secondary corpora lutea formation following exogenous gonadotropin treatment in the domestic cat and effect of passive transfer of gonadotropin-neutralizing antisera. *Theriogenology* 1996;45: 561–72.
- [16] Lawler DF, Johnston SD, Hegstad RL, Keltner DG, Owens SF. Ovulation without cervical stimulation in domestic cats. *J Reprod Fertil Suppl* 1993;47:57–61.
- [17] Gudermuth DF, Newton L, Daels P, Concannon P. Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. *J Reprod Fertil Suppl* 1997;51:177–84.
- [18] Ackermann CL, Destro FC, Volpato R, Oba E, Lopes MD. A citologia vaginal induz a ovulação em gatas domésticas? [Vaginal cytology induces ovulation in domestic cats?] Congresso Internacional de Endocrinologia, Búzios, Brazil, 2010.
- [19] Toydemir TSF, Kiliçarslan MR, Olçaç V. The effects of GnRH analogue deslorelin implants on reproduction in female domestic cats. 6<sup>TH</sup> International Symposium on Canine and Feline Reproduction & 6<sup>th</sup> Biannual EVSSAR Congress, Vienna, Austria, 2008, p. 263–4.
- [20] Graham LH, Swanson WF, Brown JL. Chorionic gonadotropin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. *Theriogenology* 2000;54:1117–31.

- [21] Root Kustritz MV. Clinical management of pregnancy in cats. *Theriogenology* 2006;66:145–50.
- [22] Volkmann DH, Kutzler MA, Wheeler R, Krekeler N, Klewitz J, Lamb SV. Failure of hCG to support luteal function in bitches after estrus induction using deslorelin implants. *Theriogenology* 2006;66:1502–6.
- [23] Goodrowe KL, Howard JG, Wildt DE. Embryo recovery and quality in the domestic cat: natural versus induced estrus. *Theriogenology* 1986;25:156.
- [24] Grabiec A, Max A, Tischner M. Parthenogenetic activation of domestic cat oocytes using ethanol, calcium ionophore, cycloheximide and a magnetic field. *Theriogenology* 2007;67:795–800.
- [25] Rascado TS, Rigatto Martins LR, Watanabe Minto BW, de Sá Lorena SE, Cruz Landim-Alvarenga FdaC. Parthenogenetic development of domestic cat oocytes treated with ionomycin, cycloheximide, roscovitine and strontium. *Theriogenology* 2010; 74:596–601.