

# The use of a slow release GnRH-agonist implant in female ferrets in season for oestrus suppression

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## Summary

The jill is a long-day breeder with a constant oestrus without mating. Persistent oestrogen production results in clinical signs of hyperoestrogenism including pancytopenia and death if untreated. As spaying is thought to be related to the development of hyperadrenocorticism, a non-invasive, safe and effective long-term treatment is needed for oestrus suppression in jills. Seven jills in oestrus were treated with a 4.7 mg deslorelin implant. Blood samples for estradiol-17 $\beta$  (E2) and progesterone (P4) determination were obtained before as well as 4 and 8 weeks after treatment; data are given as geometric mean (deviation factor, DF). Mean E2 was 280.2 pmol/L (1.7) before, 36.4 pmol/L (1.4) 4 and 21.6 pmol/L (1.1) 8 weeks after treatment ( $p < 0.0001$ ). P4 before treatment was 1.4 nmol/L (2.6), 57.8 nmol/L (1.9) on week 4 and 3.8 nmol/L (2.6) on week 8 ( $p < 0.0001$ ) indicating ovulation had occurred after implant insertion. Oestrous signs within the observation period of up to 32 months remained suppressed.

Keywords: oestrus suppression, deslorelin, induced ovulation, downregulation, ferret

## Der Einsatz eines Slow Release GnRH-Agonist Implantats bei östrischen Frettchenfähen zur Ranzunterdrückung

Die Frettchenfähe gehört zu den frühjahrsbrünstigen Tieren (long-day breeder) mit induzierter Ovulation. Die permanente Östrogenproduktion führt zu klinischen Anzeichen eines Hyperöstrogenismus mit Pancytopenie und unbehandelt zum Tod des Tieres. Da die Entstehung eines Hyperadrenokortizismus in kausalem Zusammenhang zur Kastration zu stehen scheint, ist eine nicht invasive, sichere und wirksame Langzeitbehandlung zur Unterdrückung der Ranz bei Fähen notwendig. 7 Fähen im Östrus wurden mit einem 4.7 mg Deslorelin-Implantat behandelt. Blutproben zur Bestimmung von Östradiol-17 $\beta$  (E2) und Progesteron (P4) wurden vor und 4 sowie 8 Wochen nach Implantation entnommen. Die Ergebnisse sind als geometrischer Mittelwert (Streu faktor, SF) dargestellt. Die mittlere E2-Konzentration betrug 280.2 pmol/L (1.7) vor, 36.4 pmol/L (1.4) 4 und 21.6 pmol/L (1.1) 8 Wochen nach Implantation ( $p < 0.0001$ ). Die P4-Konzentrationen vorher betragen 1.4 nmol/L (2.6), 57.8 nmol/L (1.9) in Woche 4 und 3.8 nmol/L (2.6) in Woche 8 ( $p < 0.0001$ ), was darauf hinweist, dass durch die Behandlung eine Ovulation ausgelöst wurde. Innerhalb des Beobachtungszeitraums von bis zu 32 Monaten wurde kein weiterer Östrus beobachtet.

Schlüsselwörter: Östrusunterdrückung, Deslorelin, induzierte Ovulation, Downregulierung, Frettchen

## Introduction

The female ferret (*Mustela putorius furo*, jill) is a seasonal breeder and induced ovulator, mating triggering ovulation (Carroll et al., 1985; Villars et al., 1990). As jills are 'long-day' seasonal breeders with an oestrous period

from March to August (Marshall, 1904), oestrus can persist for up to 5 months if not mated (Hammond and Marshall, 1930). However, under artificial lighting conditions, they can be induced to breed year-round (Fox and Bell, 1998). Persistent oestrogen production of the ovaries does not only result in clinical signs of oestrus like swol-

## 488 Originalarbeiten/Original contributions

len vulva and oestrous-like behaviour but also in signs of hyperoestrogenism like bilateral alopecia and pancytopenia (Cooper et al., 1985; Baumgärtner and Juchem, 1987; Fox and Bell, 1998). Animals in this state are usually presented with anaemia, anorexia, apathy, melaena and subcutaneous haemorrhage (Baumgärtner and Juchem, 1987). If recognized too late or left untreated, animals die as a consequence of bone marrow suppression, pancytopenia (Cooper et al., 1985; Baumgärtner and Juchem, 1987). Only ovulation – induced by natural mating or application of GnRH-agonists or human chorion gonadotropin (hCG) – can interrupt the vicious cycle of persistent oestrogen production by the follicles as pseudopregnancy or pregnancy is induced (Mead et al., 1988; Prohászki et al., 2010).

The classical approach to achieve contraception and avoid oestrogen intoxication is spaying. However, it seems likely that the loss of negative feedback following spaying is related to hyperadrenocorticism, a common disease in spayed and castrated ferrets (Rosenthal et al., 1993; Schoemaker et al., 2000). Manipulation of photoperiod is another, non medical option for influencing the reproductive cycle (Fox and Bell, 1998). Regarding hormonal treatment, application of progestins for reproduction control in ferrets has been described in literature as a long-term hormonal contraception (Oxenham, 1990). In dogs and cats, several side effects of this treatment, like cystic endometrial-hyperplasia-pyometra complex, hypoadrenocorticism, alopecia, diabetes mellitus, and others have been described (Romagnoli and Concannon, 2001), whereas in ferrets only progressive alopecia has been documented (Prohászki et al., 2010). Alternatively, application of a slow release GnRH-agonist implant offers an effective, safe and reversible method to surgical castration in the male dog (Trigg et al., 2001; Junaidi et al., 2003; 2007; Ludwig et al., 2009; Goericke-Pesch et al., 2010 a, b), cat (Goericke-Pesch et al., 2011) and ferret (Schoemaker et al., 2008). Treatment with slow release GnRH agonists has already been described in jills not in season (Prohászki et al., 2010). The aim of the present study was to investigate the effect of a 4.7 mg deslorelin implant on ferrets in oestrus including hormonal and behavioural changes, duration of efficacy and safety of treatment.

## Animals, Material and Methods

### Animals

Seven privately owned jills in oestrus for at least 4 weeks were presented for spaying at the Clinic in April 2009 (n = 2) and between February and May 2010 (n = 5). The animals were between 1 and 3 years old and weighed between 750 g and 850 g at initial presentation. The animals were housed in groups with other spayed or intact jills, and in one case with a surgically castrated hob. Jills were

kept indoors with restricted access to outdoor facilities (garden) under individual observation. None of the owners tried to induce ovulation manually nor used a vasectomised hob. The animals were neither pre-treated nor treated within the observation period except for the latter administration of the GnRH agonist implant. Owners were properly informed about treatment possibilities (surgical versus hormonal) for oestrus suppression. All of them chose hormonal castration with a GnRH agonist implant.

### Clinical examinations and administration of the deslorelin implant

Before inclusion in the study, all ferrets were clinically examined, especially for the presence of vulvar swelling and the mucosa for oestrogenic influence. Jills were anaesthetized with isoflurane and the GnRH-agonist implant containing 4.7 mg deslorelin (Suprelorin®, Virbac, Bad Oldesloe, Germany) was inserted subcutaneously between the shoulders. All jills were examined 4 and 8 weeks after treatment for signs of estrogenic influence and adverse reactions at the injection site. Owners were advised to regularly inspect the injection site within the first week after implant insertion. Behavioural changes and changes in the intensity of odour were noted at each consultation.

### Blood collection

Blood samples were collected from the Vena cephalica antibrachii before as well as 4 and 8 weeks after treatment into heparinized tubes. Samples were centrifuged for 10 min at 2000 rpm at 4 °C and the plasma frozen at –20 °C until analysis.

### Hormone analysis

Estradiol-17 $\beta$  (E2) and progesterone (P4) concentrations were determined by an in-house radioimmunoassay previously described in detail (Hoffmann et al. 1992). Detection limits were 7.34 pmol/L for E2 and 0.32 nmol/L for P4. Intra-assay coefficient of variation were 6.0 and 11.4%, inter-assay coefficient of variation varied between 13.1 and 13.2%, respectively.

### Statistical analysis

All data were analysed using Microsoft Excel (Windows XP; Microsoft) and the statistical software program, GRAPHPAD3 (GraphPad Sigmastat® software, release 3.5; Systat Software, Inc., San Diego, CA, USA). Due to uneven distribution of E2 and P4 concentrations, data were presented as geometric mean and deviation factor [ $x_g(DF)$ ]. To test for the influence of examination date, a nonparametric one-way analysis of variance (ANOVA/Kruskal-Wallis) for repeated measures was applied. Values were considered to be statistically significant at  $p < 0.05$ .

## Results

According to anamnesis, all animals displayed typical oestrous signs for 4–6 weeks before treatment started; clinical examination revealed no abnormalities except for a significantly swollen vulva. 4 weeks after GnRH treatment, the vulva was obviously less swollen in all animals; the owners reported an obvious decrease of vulvar swelling and intensity of odour from week 1 and 2 after treatment, respectively. The vulva was very small when examined at week 8. Food intake was increased from 5 to 7 weeks after treatment in all animals.

### Blood sampling and hormonal changes

The hormonal changes 4 and 8 weeks following treatment are shown in Figure 1. E2 concentrations were significantly ( $p < 0.001$ ) different before as well as 4 and 8 weeks after treatment. Mean E2 concentrations before treatment were 280.2 pmol/L (1.7) [range: 139.5 pmol/L – 495.5 pmol/L]. 4 weeks after treatment, the concentrations significantly ( $p < 0.001$ ) decreased to 36.4 pmol/L (1.4) [25.7 pmol/L – 64.2 pmol/L] and 8 weeks later the mean value was 21.6 pmol/L (1.1) [18.4 pmol/L – 23.9 pmol/L] that is significantly ( $p < 0.001$ ) different from pre-treatment E2 values.

P4 concentrations were significantly ( $p < 0.001$ ) different between examination dates. Mean concentrations before treatment were 1.4 nmol/L (2.6) [range: 0.32 nmol/L – 5.7 nmol/L] and significantly ( $p < 0.001$ ) increased to 57.8 nmol/L (1.9) [28.2 nmol/L – 89.8 nmol/L] 4 weeks after implant insertion. Compared to week 4, mean P4 values [3.8 nmol/L (2.6); range: 1.2 nmol/L – 3.2 nmol/L] were significantly ( $p < 0.001$ ) decreased in week 8.

### Side effects

No local reaction occurred except for a slight swelling at the injection site in one jill by one owner within the first 2–3 days after treatment. Treatment related negative side effects were not observed in the 7 treated animals. How-

ever, behavioural changes like neck biting of another ferret of the familiar group and temporary biting the owner ( $n = 1$ ) were reported.

### Duration of efficacy

No oestrous signs were observed after administration of the implant until the end of observation period (March 2012) indicating that oestrus is suppressed at least between 22–35 months depending on the individual ferret.

## Discussion

All jills in this study showed typical clinical oestrous signs like a swollen, enlarged vulva (Hammond and Marshall, 1930), an intensive odour of skin oils and urine (Quesenberry and Carpenter, 2004) and high E2 concentrations in the peripheral plasma. Following administration of the GnRH agonist, the vulva clearly decreased in size within 1–2 weeks indicating that ovulation had occurred (Hammond and Walton, 1934). This observation was verified by a significant decrease of E2 concentrations and a significant increase of P4 in week 4 whereas P4 concentrations were similar to those reported after natural mating or induction of ovulation (Blatchley and Donovan, 1972, 1976; Heap and Hammond, 1974). In week 8, the vulva was very small and low E2 as well as P4 concentrations were measured, similar to ferrets out of season. If pseudopregnancy would have been induced by ovulation, a return to oestrus should be expected 2–8 weeks later (Marston and Kelly, 1969; Lindeberg, 2008). This, however, was not the case in the treated animals most likely indicating hormonal downregulation of ovarian endocrine function, although FSH and LH concentrations were not measured. In contrast to this study, anaesthesia is not necessary for insertion of the implant under clinical conditions.

Regarding side effects, only a slight short-term local reaction (swelling) was observed in one of 7 ferrets following insertion of the implant. However, behavioural changes may occur and owners have to be informed about it. Neck biting is the most common aggressive behaviour in ferrets (Poole, 1966). None of the jills returned to oestrus until March 2012 indicating that ovarian function can be suppressed for at least 22 to 35 months (three breeding seasons). This observation is in good agreement with the duration of efficacy of deslorelin in anoestrous jills for suppression of ovarian activity [698 (122) days, data presented as geometric mean and deviation factor] (Proháczik et al., 2010) and in male ferrets as an alternative to surgical castration (> 173 days) (Schoemaker et al., 2008).

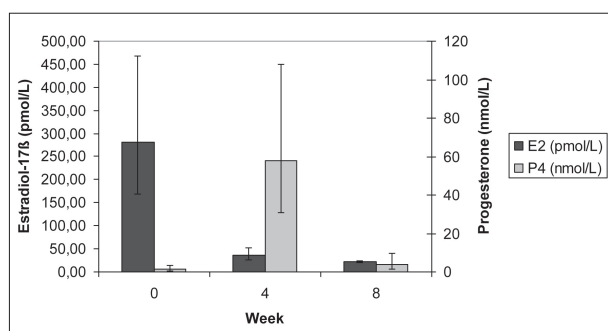


Figure 1: Hormonal changes 4 and 8 weeks following treatment with a 4.7 mg deslorelin implant in 7 jills in season. Progesterone (nmol/L) and estradiol-17 $\beta$  (pmol/L) concentrations are presented as geometric means and deviation factors.

## Conclusion

Treatment with a GnRH-agonist slow release implant containing 4.7 mg deslorelin is a suitable alternative for

## 490 Originalarbeiten/Original contributions

spaying jills in season. Suppression of oestrus (vulvar swelling, musky odour and behavioural changes) may last more than three breeding seasons (long-term contraception) making the implant an attractive alternative to all current approaches. As the implants are licensed for the use in male dogs only, effects and potential side effects have to be carefully explained to the owner.

### Utilisation d'un implant d'agoniste de GnRH à relargage lent chez les femelles furet pour la suppression des chaleurs

Les femelles furets font partie des animaux aux chaleurs printanières (long-day breeder) avec ovulation induite. La production permanente d'oestrogènes conduit à des signes d'hyperoestrogénisme avec pancytopénie et, si elle n'est pas traitée, à la mort de l'animal. Comme l'apparition d'un hyperadrenocorticisme semble en rapport causal avec la castration une méthode non invasive, sûre et de longue durée pour la répression des chaleurs est nécessaire. Sept femelles furets en chaleur ont été traitées avec un implant de 4.7 mg de Désloreline. Des échantillons sanguins pour déterminer l'oestradiol-17 $\beta$  (E2) et la progestérone ont été prélevés 4 et 8 semaines après l'implantation. Les résultats moyens géométriques sont présentés. La concentration moyenne E2 se montait à 280.2 pmol/l (1.7) avant le traitement, à 36.4 pmol/l (1.4) 4 semaines et 21.6 pmol/l (1.1) 8 semaines après l'implantation ( $p < 0.0001$ ), ce qui démontre qu'une ovulation a été induite par le traitement. Durant le temps d'observation il n'a pas été observé de signe de chaleurs.

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### L'uso di impianto agonista GnRH a lento rilascio nei furetti in calore per sopprimere l'estro

Il furetto fa parte degli animali che vanno in calore molto presto (long-day breeder) con un'ovulazione indotta. La costante produzione di estrogeni porta a segni clinici di un iperestrogenismo con pancitopenia e se non curata alla morte dell'animale. Poiché sembra che lo sviluppo di un iperadrenocorticisme sia in relazione causale con la castrazione, è necessario un trattamento non-invasivo, sicuro ed efficace e a lungo termine per eliminare l'estro nel furetto. 7 furetti in estro sono stati trattati con un impianto di 4.7 mg di desloreline. I campioni di sangue per la determinazione di estradiolo-17 $\beta$  (E2) e di progesterone (P4) sono stati prelevati prima, a 4 e a 8 settimane dopo l'impianto. I risultati sono espressi come media geometrica (coefficiente di variazione). La concentrazione media di E2 era di 280.2 pmol/L (1.7) prima, 36.4 pmol/L (1.4) a 4 e 21.6 pmol/L (1.1) a 8 settimane dopo l'impianto ( $p < 0.0001$ ). Le concentrazioni di P4 sono state prima di 1.4 nmol/L (2.6), 57.8 nmol/L (1.9) alla settimana 4 e 3.8 nmol/L (2.6) alla settimana 8 ( $p < 0.0001$ ). Ciò indica che grazie al trattamento l'ovulazione è stata innescata. Durante il periodo di osservazione fino a 32 mesi, non è stato notato alcun estro.

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