Slow release GnRH-agonist implant in female ferrets

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

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Introduction
The female ferret (Mustela putorius furo, jill) is a seasonal breeder and induced ovulator, mating triggering ovulation (Carrol 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 oestrous like swol-
len vulva and oestrous-like behaviour but also in signs of hyperoestrogenism like bilateral alopecia and pancyclopenia (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, panmyelophthisis (Cooper et al., 1985; Baumgärtner and Juchem, 1987). Only ovariectomy – 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 (Meade et al., 1988; Proháczik 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 (Proháczik 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; Junaaidi 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 (Proháczik 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 vasectomy 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 antebrachii 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-17β (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 Sigmatstat® 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 [\(\bar{x}_g(DF)\)]. 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. However, 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).

**Conclusion**

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

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