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Reversibility of germinative and endocrine testicular function after long-term contraception with a GnRH-agonist implant in the tom—a follow-up study

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ABSTRACT

A significantly reduced gonadotropin and testosterone secretion is a well-described result of long-term administration of GnRH agonists in the male dog and cat. To date, no data are available about the duration of efficacy and the reversibility of treatment-induced effects after long-term treatment with a 4.7 mg deslorelin implant. Seven healthy male European Shorthair cats (3.2 ± 0.5 kg, 1–6 years) were treated with a 4.7 mg deslorelin implant. Blood samples (testosterone, T), testicular volume, penile spines, and mating behavior were recorded once weekly. Considering T > 0.5 ng/mL as the biological endpoint, mean duration of efficacy was 78.8 ± 12.9 weeks (range: 61.7–100.7 weeks) with T concentrations increasing rapidly after the last T less than 0.1 ng/mL (basal) ($P < 0.0001$), and pretreatment T concentrations being reached after 3 weeks. Testicular volume rapidly increased after the first increase of T ($P < 0.001$) with pretreatment testicular volume being reached after 6.9 ± 3.4 weeks (5–11 weeks). "Normal" libido reoccurred 88.7 ± 12.4 weeks after treatment, and "normal" mating behavior was observed even later. Fertile matings occurred 7 to 42 weeks after the last T less than 0.1 ng/mL with a mean of 4.0 ± 0.0 kittens, and 13.6 to 47.6 weeks afterwards testicular histology revealed normal spermatogenesis. The present data confirm that the use of slow-release GnRH-agonist implants containing deslorelin in tomcats represents an effective and safe reversible alternative for long-term contraception; however, as number of animals is low, further fertility trials are recommended.

1. Introduction

Downregulation of pituitary GnRH receptors and consequently, a significantly reduced gonadotropin and testosterone secretion is a well-described result of long-term administration of GnRH agonists in the male dog [1–7] and cat [8–11]. Currently, a slow-release implant containing either 4.7 or 9.4 mg deslorelin is available on the market. The registered indication is to achieve "a temporary infertility in adult healthy male dogs and ferrets". Whereas the duration of efficacy of the 4.7 mg implant is at least 6 months, the effects of treatment with a 9.4 mg implant expire after a minimum of 12 months in the male dog according to the manufacturers' product information (Suprelorin; Virbac,

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Carros Cedex, France; http://www.ema.europa.eu/ema/). Several studies reported about the reversibility of the effects induced by slow-release GnRH-agonist implants in dogs including testosterone concentrations, testicular volume, and semen quality [1–3,5,6,12,13]. To date, there is only one study reporting reversibility of treatment-induced effects in toms [10]; however, as the 4.7 mg deslorelin implants were removed 4 months after application, these data only refer to short-term contraception [10].

To our best knowledge, no data are available about the long-term use of deslorelin implants, the duration of efficacy and the reversibility of treatment-induced effects after long-term treatment with a 4.7 mg deslorelin implant in toms. The aim of the present study was to address these questions by investigating the endocrine and clinical changes, the duration of efficacy of a single treatment with one 4.7 mg deslorelin implant and the reversibility of treatment-induced effects with special respect to the return of fertility in the male cat. This study is a follow-up study of seven toms from our previous study that described treatment-induced effects and hormonal changes up to week 36 in 10 toms [8].

2. Materials and methods

Animal experimentation was approved by the local authority (Ethic Commission of Veterinary Faculty of the Trakian University, Stara Zagora, Bulgaria).

2.1. Animal selection

As this is a follow-up study, animal selection and housing were the same as reported in our previous study [8]: Seven healthy male European Shorthair cats (3.2 ± 0.5 kg, 1–6 years) assigned to this experiment were followed until the end of the study. The remaining three of the 10 toms from our previous data were not followed because of different reasons. Five healthy female cats of the same breed served as teaser queens for testing sexual behavior (mounting, mating). They lived in two sex-separated collectives. All male animals were treated on April 11, 2009.

2.2. Experimental design

The trial was designed as a monocentric, non-randomized and nonblinded study, and described in detail in Goericke-Pesch et al. [8]. Briefly, the observation period was divided into four experimental periods (EPs) of which EP I to III have been previously described [8]:

EP I: acclimatization phase;
EP II: pretreatment period of 4 weeks;
EP III: treatment period, on Day 0, all toms received a Suprelorin implant containing 4.7 mg deslorelin (Virbac) by subcutaneous injection into the neck according to the manufacturer's instructions. Blood samples and testicular measurements were taken once weekly, and the presence of penile spines was investigated at 4-week intervals. Additionally, sexual activity (libido, mounting, and mating) of the toms in the presence of an estrous queen was observed and recorded once per week; and
EP IV: posttreatment period, after the end of efficacy and until typical tomcat behavior was observed, blood samples were collected for a minimum of 6 weeks, and andrological examination was performed once weekly. Afterward, toms either mated a proven fertile queen (n = 4) until the queen got pregnant, and/or they were surgically castrated (n = 5 thereof n = 2, toms No. 2 and 10, mated successfully, and were castrated later on).

2.3. Blood sampling and hormone assays

Blood samples were collected via puncture of the jugular vein into lithium heparin coated plastic tubes; the plasma was obtained after centrifugation (1000× *g*, 5 minutes, +4 °C), and stored at −22 °C until analysis [8]. Testosterone (T) concentrations were measured by specific in-house RIAs as previously described [14–16]. Intra- and interassay coefficients of variation were between 7.8 and 9.0.

2.4. Andrological examination

Andrological examination included visual examination of the penis for the presence of penile spines (present or not present and if present, subjective evaluation small-sized or normal-sized spines). Additionally, measurement of testicular length (A), width (B), and depth (C) (mm; including scrotal skin) were obtained to calculate testicular volume (mm^3) using the equation of a modified spherical volume (mm^3) = $4/3 \cdot \pi \cdot (½a \cdot ½b \cdot ½c)$ [8].

2.5. Assessment of gonadal downregulation, sexual behavior, and duration of efficacy

Sexual behavior (libido, mounting, and mating) was assessed and scored as previously described [1]. Briefly, it was recorded if the tom was interested in the queen, if and in what time (immediately, slowly, and hesitantly) he tried to mount and mate her resulting in a scoring from 0 to +++ [1]. For observations, all toms were kept individually together with an estrous queen for about 1.5 hours once a week; after each tom displayed its behavior, it was replaced by the next one. The individual duration until sexual behavior occurred was not recorded in detail. The queens used for testing were usually in natural estrous; only very rarely, no estrous queen was available. In these cases, an anestrous queen was used.

As previously described, T concentrations below 0.1 ng/mL were considered as an indication of full downregulation of testicular function [8]. For further description of data, the date when the last basal T concentration was measured was defined as week 0. But as intact toms might also occasionally have T less than 0.1 ng/mL [17], definition of a biological endpoint with T certainly pointing to testicular steroidogenesis was necessary. Therefore and according to the literature in dogs [6], T > 0.5 ng/mL was considered as the biological endpoint, and used for calculation of the duration of efficacy.