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Azoospermia with variable testicular histology after 7 months of treatment with a deslorelin implant in toms

R. Novotny [a,*], R. Vitasek [a], A. Bartoskova [a], P. Cizek [b], P. Prinosilova [c], K. Novakova [a]

[a] Department of Reproduction, Clinic of Dog and Cat Diseases, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[b] Department of Anatomy, Histology and Embryology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[c] Department of Genetics and Reproduction, Veterinary Research Institute, Brno, Czech Republic

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ABSTRACT

The main aim of the study was to assess whether the longer use of a GnRH-agonist implant (deslorelin 4.7 mg, Suprelorin) in toms would lead to the suppression of spermatogenesis comparable with histologic appearance in juvenile animals as was previously described in dogs. The other aims were to monitor the progression of the testes size decrease and development of azoospermia 5 to 7 months after treatment with a GnRH-agonist implant. In animals, 5, 6, and 7 months after GnRH-agonist implant insertion, variable histological appearance of germinal epithelium was found, when tubules with elongating spermatids, round spermatids, spermatocytes, and spermatogonia as the most developed germinal cells were found in each group of toms. In all male cats, 5, 6, and 7 months after implant insertion, testosterone concentrations and testes size significantly differed between the first and the last visit. All animals, except one tom castrated 5 months after implant insertion, developed complete azoospermia. However, in this tom, all spermatozoa were immotile. Treatment with the subcutaneous GnRH-agonist implant was well tolerated, and no treatment-related adverse effects were noted. These results reported the efficacy of 4.7-mg deslorelin implant (Suprelorin) during its 7 months of use. The complete azoospermia confirms its contraceptive effect. However, the histologic evaluation revealed a great individual variability in the degree of spermatogenic suppression. The question as to whether spermatogenesis in toms can be suppressed in all males to the level of spermatogonia/primary spermatocytes after prolonged exposure to deslorelin has yet to be answered.

1. Introduction

Contraceptive effect of subcutaneous implants releasing a GnRH-agonist has already been confirmed in male dogs [1–11] and cats [10,12–15]. In dogs and toms, the long-term effect of a GnRH-agonist implant led to a significant decrease in testosterone levels and testes size [1–7,9–15], and spermatogenesis and semen quality were significantly impaired [1,2,9,13,15]. The contraceptive effect of GnRH-agonists in dogs and toms was proven to be completely reversible [4,6,8,9,11,13,15]. Nevertheless, the histologic appearance of the seminiferous epithelium after suppression of spermatogenesis in toms was different to that of dogs. Five-month exposure to the GnRH-agonist, azagly-nafarelin, released from a subcutaneous implant

* Corresponding author. Tel./fax: +420 541 562 322.
E-mail address: novotnyr@vfu.cz (R. Novotny).

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caused spermatogenic arrest in dogs at the level of spermatogonia/primary spermatocytes [6] similar to results obtained earlier in dogs after the administration of a slow-release buserelin implant [11]. A comparable level of spermatogenic suppression was also found in boars 23 weeks after the insertion of a deslorelin implant [16]. In dogs, the histologic appearance of the seminiferous epithelium of suppressed males resembles that of juvenile animals [6]. Contrary to that, histologic examination of the seminiferous epithelium in toms 4 months after the administration of a subcutaneous implant containing the GnRH-agonist deslorelin revealed this level of suppression only in one animal, whereas another tom had the majority of tubules containing round spermatids as the most developed germ cells present, and the other two toms had the majority of tubules with elongating and elongated spermatids, respectively [13]. Similar histologic findings with variable atrophy of germinal epithelium in male cats after 20 weeks of treatment with an implant containing 4.7-mg deslorelin were recently achieved also by Goericke-Pesch et al. [14]. The question whether longer exposure to the GnRH-agonist would lead to the suppression of spermatogenesis to the level of spermatogonia/primary spermatocytes in toms as in males of other species still needs to be clarified.

The main aim of the study was to assess whether the longer use of a GnRH-agonist implant (deslorelin 4.7 mg, Suprelorin) in toms would lead to the suppression of spermatogenesis comparable with histological appearance in juvenile male cats as was previously described in dogs. The other aims were to monitor the progression of the testes size decrease and development of azoospermia 5 to 7 months after treatment with a GnRH-agonist implant.

2. Materials and methods

The study was approved by the Ethical Committee, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic; Approval No. 31 to 2013 and by the Ministry of Education, Youth and Sports; Approval No. MSMT-22385/2013 to 10.

2.1. Animal selection

Twenty-two indoor male cats from private owners, referred to the Small Animal Clinic, Department of Reproduction, University of Veterinary and Pharmaceutical Sciences Brno (49.22 N and 16.60 E) for fertility control, were put under observation. All tomcats were given a general examination before entering the study and were found to be clinically healthy. Because of presumed influence of the day length on testis function in toms, only indoor animals living on a relatively stable photoperiod (at least 14 hours per day) were included in the study. All owners were informed about the procedures performed during the study and gave their informed consent.

2.2. Experimental design

Animals were divided into three experimental groups: group 1, group 2, and group 3. Group 1 consisted of 12 sexually mature toms (12–48 months; 4.23 ± 0.54 kg). Animals with typical sexual characteristics and behavior such as male odor and urine marking were assigned to the group. Of 12 toms, nine males were European Shorthair, one Shorthair Chinchilla, one Devon Rex, and one Maine Coon Cat. After the general examination, the following procedures were performed: measurement of testicular length and width and collection of blood and semen samples. At the end of the first visit, each of the 12 toms was given one deslorelin implant subcutaneously in the region of right shoulder blade. The follow-up examinations were performed 5, 6, and 7 months later for four toms each. At the end of the follow-up examination, orchiectomy was performed, and testes were submitted to histologic evaluation.

Group 2 consisted of five sexually mature European Shorthair toms (14–23 months; 4.17 ± 0.89 kg) that served as an adult control group for the histologic examination. Spermatogenesis in all males was confirmed by semen collection, toms were then castrated, and testes were submitted to histologic evaluation.

Group 3 consisted of five prepubertal European Shorthair male cats (14–16 weeks; 1.25 ± 0.06 kg) that served as a control group for the histologic examination. All animals had no visible penile spines present and the prepuce still not separated from the penis, as the indirect evidence of testosterone absence. All toms were castrated, and testes were submitted for histologic evaluation.

2.3. Blood sampling and hormone assay

For tomcats from group 1, blood samples were collected from the cephalic vein into plastic tubes with blood clotting granules for the assessment of testosterone concentrations. Blood samples were collected before (T0) and 4 hours after (T4) the intramuscular administration of 250 IU of hCG (Pregnyl; Organon, Oss, the Netherlands) [17]. Serum was obtained after centrifugation, and testosterone concentration was measured by chemiluminescence (Modular/Elecsys; Roche, Basel, Switzerland). The detection limit was set to 0.03 ng/mL.

2.4. Measurement of the testicular size

Measurement of the testicular length (a) and width (b; mm; including the scrotal skin) in the left and right testicle was performed with a caliper. The testicular size was calculated, applying the equation: area (mm^2) = ½a · ½b · π. The absolute testicular size was calculated by the addition of the values for the left and right testicle.

2.5. Semen collection

To obtain semen from animals in the study, an electroejaculator with a 0.95-cm-diameter probe with two longitudinal electrodes (Electroejaculator e320; Minitube) was used. The procedure was performed under general anesthesia with medetomidine (Cepetor; CP-Pharma, Burgdorf, Germany), 80 μg per kg, IM and ketamine (Narketan, Vétoquinol, Nymburk, Czech Republic), 5 mg per kg, IM. If necessary, the depth of anesthesia was increased with