ABSTRACTS

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Onset of sterility following administration of a 4.7 mg deslorelin implant in adult male dogs

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OBJECTIVES AND METHODS: Deslorelin implants are being increasingly used for the control of fertility of adult male dogs in most European countries as well as outside of Europe. Despite a well known efficacy of deslorelin on induction of (reversible) sterility due to a temporary pituitary block caused by a prolonged positive feed-back on the pituitary, little is known about the timing of such effect in carnivores. Our study was designed to establish how long it takes for a dog to become sterile following treatment with a 4.7 mg implant of deslorelin.

Six privately owned dogs of various breeds and body weight (an 18 mo old, 14.2 kg bw Tibetan terrier; an 8½ yr old, 7.8 kg bw Yorkshire terrier; an 8 yr old, 11.3 kg bw mongrel; 3 Dobermans of 2½, 6 and 6 yr of age and 40.2 ±2.4 kg bw) were used for this study. Dogs were referred to the Veterinary Teaching Hospital of the University of Padova (Italy), with the request to control aggressiveness and/or fertility. The following protocol was used for each dog on the first day of the study (Day 0): a) collection of data related to signalment, general/reproductive history, general physical/reproductive exam; b) collection of a blood sample for hematology, serum biochemistry and assay of basal serum testosterone (T) concentration; c) collection and evaluation of semen; d) intravenous injection of 50 mcg gonadorelin GnRH (Fertagyl™, Intervet); e) second blood collection 60 minutes later to assay post-GnRH serum T concentration; f) administration of one 4.7 mg implant of deslorelin (Suprelorin™, Virbac) between the shoulder blades. Owners were instructed to bring their dogs for blood and semen collection every 2 weeks until the observation of two consecutive semen collections in which the dog could be considered infertile. At the end of the study owners were asked to fill out a behavioral questionnaire concerning whether and how the dog had changed his attitude towards owner, family members, other male and female dogs, bitches in heat, appetite and general physical activity.

Semen collection and evaluation was performed routinely with the presence of a bitch in heat collection was continued for at least 5 minutes even if no semen was being produced. In the event of aspermia, a urine sample was collected and the urine sediment analyzed for presence of sperms, to rule out retrograde ejaculation. Progressive motility was evaluated under light microscopy at 10X, morphology of 100 spermatozoa was assessed on a Diff Quik-stained semen smear under light microscopy at 100X. The number of sperm cells was counted using a Bürker chamber. The study ended when complete sterility was achieved, based on presence of <10 million of progressively motile sperms (PMS) and semen volume <0.5 cc.

Blood samples were collected in plain and EDTA-vacutainer tubes. Following haematology (ADVIA Haematology counter system, Siemens Medical Solutions Diagnostics, s.r.l., Bayer, Germany) and serum biochemistry (Hitachi 912 Automatic Analyzer, Roche Diagnostics GmbH, D-68298 Mannheim, Germany), serum samples were aliquoted and stored frozen. Testosterone was assayed using a chemiluminescence system (Immulite, Medical System, Genova, Italy). Results were analyzed using analysis of variance for repeated measures, grouping data in the following classes of distance from the day of treatment (Day 0): class 1 = day 0; class 2 = day 9-17; class 3 = day 23-32; class 4 = day 37-47; class 5 = day 51-60; class 6 = day 64-75; class 7 = day 84-89. Classes of distance from day 0 were considered as the independent factor. Pearson’s correlation coefficients (considered significant when >0.4) were used to investigate correlation between treatment and fertility parameters.

RESULTS: General clinical as well as fertility parameters were within normal limits for all dogs at the onset of the study. Semen motility and total number of sperms initially increased and then decreased, semen morphology was unaffected, and semen volume showed a gradual and continuous decrease. PMS (%) was 65±14, 80±2, 65±14, 56±13, 32±15 and 10±10 during periods 1 through 6, respectively; total number of sperm was 620±160, 550±120, 750±60, 300±50, 100±20 and 0 millions during periods 1 through 6, respectively; percentage of abnormal sperms fluctuated between 12% and 10% throughout the study periods; semen volume was 6.0±1.8, 5.8±1.4, 5.2±2.0, 2.2±1.4, 1.0±0.6 and 0 cc during periods 1 through 6, respectively. Serum T concentration was 5.3±1.6, 4.5±1.2, 2.5±0.6, 0.9±0.3, 0.7±0.3 and <0.2 ng/ml during periods 1 through 6, respectively. The treatment caused a significant (P<0.05) decrease of serum T as well as sperm motility. Complete sterility (based on presence of <10 million of progressively motile sperms (PMS) and semen volume <0.5 cc) was achieved on post-treatment days 70, 84, 60, 23, 51 and 40 for dogs 1 through 6, respectively. All dogs were still considered fertile at their previous biweekly check based on percent motility and total sperm count (Table n° 1), and their semen quality did not change later. Sterility was achieved at 54±21 days post-treatment. All dogs were aspermic at a subsequent semen collection.
Table 1: Dog identification and age, days post-treatment (post-Tx), semen volume, % progressive motile sperm (PMS) and total number of spermatozoa in the ejaculate (t-count) at the last semen collection in which the dog was considered fertile, and at the first semen collection in which the dog was considered sterile, following administration of a 4.7 mg implant of deslorelin.

<table>
<thead>
<tr>
<th>Dog ID</th>
<th>Age (yr, mo)</th>
<th>Days post-Tx (semen vol., PMS, t-count)</th>
<th>Days post-Tx (semen vol., PMS, t-count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>59 (3.0 cc, 60%, 247 millions) = fertile</td>
<td>70 (0.3 cc, 2%, 76.8 millions) = sterile</td>
</tr>
<tr>
<td>2</td>
<td>8.6</td>
<td>70 (0.5 cc, 50%, 82.0 millions) = fertile</td>
<td>84 (0.05 cc, 50%, 7.8 millions) = sterile</td>
</tr>
<tr>
<td>3</td>
<td>8.0</td>
<td>41 (2.0 cc, 70%, 18.4 millions) = fertile</td>
<td>60 (0.05 cc, 30%, 3.5 millions) = sterile</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>9 (7.0 cc, 80%, 1512 millions) = fertile</td>
<td>23 (0 cc) = sterile</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>44 (0.2 cc, 60%, 67 millions) = fertile</td>
<td>51 (0 cc) = sterile</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>28 (6.0 cc, 80%, 376 millions) = fertile</td>
<td>40 (0.03 cc, 50%, 0.96 millions) = sterile</td>
</tr>
</tbody>
</table>

CONCLUSIONS: In our study, adult male dogs with normal semen quality administered a 4.7 mg deslorelin implant showed improvement of some of their seminal parameters (motility and total count) during the first month post-treatment, followed by a progressive decline of most of the seminal parameters considered (motility, total count, semen volume, serum T) during the subsequent 3 months. Semen morphology was unaffected, but all dogs became eventually aspermic. Fertility was still within normal limits 42±22 days post-treatment (range 9-70 days) while complete sterility was achieved at 54±21 days post-treatment (range 23-84 days). Our results a) confirm efficacy of deslorelin in causing (temporary) sterility in male dogs (1,2), b) confirm and provide details about endocrine and seminal parameters involved in this process, and c) contribute to define the interval between treatment and achievement of complete sterility. Practitioners should be aware that such interval may be longer than 2 months in some cases, and that fertility may actually be increased during the first 2-4 weeks post-treatment.

(2) Junaidi A, Williamson PE, Martin GB, Stanton PG, Blackberry MA, Cummins JM, Trigg TE. Pituitary and testicular endocrine responses to exogenous gonadotrophin-releasing hormone (GnRH) and luteinising hormone in male dogs treated with GnRH agonist implants, Reproduction Fertility and Development, 2007;19 (8):891-8