SEMEN IMPROVEMENT IN OLIGOZOOSPERMIC DOGS AFTER TREATMENT WITH SPEMAN®

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Summary

The aim of the present investigation was to evaluate the effect of Speman®, a well-known ayurvedic proprietary preparation, in male dogs suffering from infertility associated with oligozoospermia. Twenty-three dogs were diagnosed with oligozoospermia by semen evaluation and a treatment with Speman® was prescribed for a period of 180 days. During this period, three control semen evaluations at 60 day intervals were made. No adverse effects were reported. Semen volume, concentration, total number of sperms per ejaculation, viability, total motility and percentage of morphologically normal spermatozoa increased significantly (P<0.05) in all treated dogs. As a formulation of plant origin, Speman® may successfully improve the sperm quality in infertile male dogs due to oligozoospermia.

Key words: dog, infertility, oligozoospermia, semen improvement, Speman®

INTRODUCTION

Oligozoospermia is an important clinical condition, manifested with decrease in the numbers and density of spermatozoa produced by testes throughout the reproductive life. Clinically, oligozoospermia is considered one of the most prevalent causes of male infertility in humans (Haslett et al., 2002), which pathophysiology remains unclear (Pramanik, 2007). No specific drug has been so far discovered as a treatment option for the condition. In the last decade, extensive research has been carried out on utilising natural sources in oligozoospermia treatment (Mucram et al., 2013).

Recent studies showed that 15–20% of male dogs used for breeding are affected by subfertility (Domoslawska et al., 2019). The major problems are diagnosed by seminal analysis and similarly to humans, are related to poor semen quality. In the infertile dogs, sperm concentration, percentage of spermatozoa with normal morphology, and the majority of most evaluated motility parameters are significantly lower than in fertile dogs (Rijselaere et al., 2007; Domoslawska et al., 2013).

Performing semen evaluation in dogs, concentration of sperm cells is usually
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measured, but it has little value as an indicator being inversely related to the volume of the ejaculate (Root Kustritz, 2007). For this reason, total number of spermatozoa in the ejaculate is calculated and in dogs it must be greater than 300 million (Power, 1963).

Semen improvement in male stud dog is a field of permanent interest (Schäfer-Somi, 2015). Investigations of different oral supplements have been made with this regard. Most often, supplementation of vitamin E alone (Hatamoto et al., 2006; Rocha et al., 2009) or in combination with vitamin C (Lopes-Santiago et al., 2012) were used for significant improvement of ejaculate volume, progressive motility and decreasing of total sperm pathology. The efficacy of a commercially available nutraceutical diet was also tested in male dogs and it was found out that motility percentage, semen volume, concentration and total number of sperms per ejaculation were significantly increased (Ciribe et al., 2018).

Speman® is a formulation of plant origin developed by The Himalaya Drug Company (Makali, Bangalore) with no side effects, which has been tested in humans with oligozoospermia, asthenozoospermia, enlarged prostate and azoospermia (Kadhem & Al-Ani, 2013). It is a mixture of extracts from Withania somnifera, Asteracantha longifolia, Lactuca scariola, Mucuna pruriens, Parmelia parlatia, Argyreia speciosa, Tribulus terrestris, Leptadenia reticulate and Suvarnavang (Mucram et al., 2013). There are also reports about the beneficial effects of Speman® on the gametogenic as well as androgenic functions of the testes in humans and animals (Kunaiah, 1966; Jadhav & Bhaga, 1971; Bhatnagar, 1973; Khaleeluddin et al., 1973; Subbarao et al., 1973). In men, the active ingredients of Speman are given by daily oral administration for a period of 4 to 6 months (Kadhem & Al-Ani, 2013). To our knowledge, there are no reports about the effect of Speman® in oligozoospermic male dogs. Therefore, the aim of this study was to evaluate for the first time the beneficial effect of Speman® on improvement of the sperm quality in infertile dogs due to oligozoospermia.

MATERIALS AND METHODS

Experimental animals and management

Twenty three, private-owned, male dogs from different breeds (4 German Shepherds, 1 English pointer, 1 English cocker spaniel, 1 Samoyed, 2 Chow chows, 2 German hunting terriers, 4 Cane corso, 1 Kurzhaar, 2 Bulgarian hunting dogs, 1 East Siberian laika, 2 Bullterriers, 1 American cocker spaniel and 1 Golden retriever), aged 3–7 years and weighing 10–47 kg were presented at the Small Animal Clinic of the Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria. Eighteen of the animals included in this evaluation had a previous history of 1–3 infertile matings and the owners requested semen analysis. The other five dogs were presented for routine breeding soundness examination.

Clinical, blood laboratory and ultrasonographic examinations

Each dog was subjected to a general examination with history, clinical and laboratory evaluations and ultrasonographic evaluation of the testicles, epididymes and the prostate gland. The haematology parameters were determined on an automated haematological analyzer BC-2800 Vet (Mindray, China). The differential blood cell counts were determined on
Giems-stained blood smears and the absolute counts of neutrophils, lymphocytes, eosinophils, basophils and monocytes were calculated. The biochemical parameters were assayed on a biochemical analyzer BA 88 (Mindray, China). Ultrasonography was done on a Mindray DC-6 Vet, China and 6.5 MHz convex transducer. Only dogs with a normal clinical, haematological, biochemical profile and without ultrasonographic pathology of the male genitalia were included in the evaluation.

**Semen collection, processing and evaluation**

Prior to semen collection, an abstinence period of minimum 10 days was provided. Semen was collected in all males by digital manipulation with a latex cone and a sterile plastic tube attached to the end in the presence of a teaser bitch to provide stimulation. Semen was collected non-separately until the end of ejaculation and immediately after that was transferred to the laboratory for analysis. Seminal quantitative and qualitative parameters (volume, concentration, total number of sperm per ejaculation, viability, motility and morphology) were evaluated.

The volume was measured by a graduated pipette. Sperm concentration (×10⁶/mL) was determined by a Photometer SpermaCue (Minitüb, Germany) and the total number of spermatozoa per ejaculation was calculated as concentration multiplied by volume.

The sperm viability was assessed by mixing 5 μL of semen with 5 μL of eosin-nigrosin and allowed to air dry. At least 200 cells were counted under a light microscope and oil immersion at magnification of 400×. Sperm cells that were unstained (white) were accepted as alive, whereas stained (pink or red coloration) were considered to be dead.

Total motility was estimated by microscopic examination using Motic Image Plus Digital System (Motic China Group Ltd, 2001–2004). Before examination, semen samples were gently mixed and a 5 μL drop was placed on a slide pre-warmed at 37 °C, covered with a 20×20 mm cover slip and observed at 200–400× by a qualified operator. The average value of three consecutive observations from at least of five different microscopic fields was calculated as a final motility (Ax et al., 2000).

To evaluate sperm morphology, at least 200 sperm cells were evaluated in semen samples after Haemacolor® staining (Merck KGaA). A 5 μL aliquot of fresh canine semen was placed on the slide, smeared, fixed with methanol, stained with the two stain components, rinsed with distilled water and air dried. Slides were examined by a light microscope at magnification of 400× and the sperm cells were assessed for normality (normal shape and normal structure).

In order to exclude retrograde ejaculation, urine sample was collected by transabdominal puncture of the urinary bladder. After centrifugation, the pellet from the bottom of the tube was examined under a light microscope at a magnification of 400×. Dogs without presence of sperm cells in the urine and with total number of spermatozoa in the ejaculate lower than 300 million were diagnosed as oligozoospermic and a treatment with Speman® at a daily dose of 1 tablet per 10 kg was prescribed for a period of 180 days. For dogs above 20 kg body weight, the dose was separated into two equal parts with an 12-hour interval. Additionally, semen collection and evaluation of the dogs was performed on days 60, 120 and 180.
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Statistical analysis

The results were processed by statistical software Statistica version 7.0 (StatSoft, Inc., Tulsa, OK, USA). All data are presented as the mean ± SD and were first checked for normality. The results were analysed using ANOVA for repeated measures followed by Tukey’s multiple comparisons test and a value for P<0.05 was considered significant.

RESULTS

Table 1 shows semen volume, concentration, total number of sperms per ejaculation, viability, total motility and percentage of morphology normal spermatozoa on days 0, 60, 120 and 180 of the Speman® treatment.

Semen volume increased significantly (P<0.05) from day 0 value of 4.55±0.41 mL to 5.13±0.55 mL even on day 60, which continued to attain the maximum value of 7.91±0.11 mL on treatment day 180. Concentration of spermatozoa was slightly increased from 55.43±13.12 ×10⁶/mL at day 0 to 61.81±15.21 ×10⁶/mL at day 60 with statistically significant differences (P<0.05) was observed on day 120 and especially on day 180, when the value of this parameter was twice higher (118.12±20.12) compared with day 0.

The total number of sperms per ejaculation showed a pattern of significant (P<0.05) elevation from 272.13±18.11 ×10⁶ total ejaculated at day 0 to 297.14±17.14 ×10⁶ total ejaculated at day 60 and from 550.23±24.11 ×10⁶ total ejaculated at day 120 to 913.34±23.08 ×10⁶ total ejaculated at the end of treatment (day 180). The percentage of viability also increased significantly (P<0.05) from 87.14±2.53 at day 0 to 89.15±1.89 and 91.12±2.08 at days 60 and 120 respectively, then attained 92.11±2.13 at day 180, but without significant difference vs day 120. A mild increase in percentage of sperm total motility was found from day 0 to day 60 (80.04±3.51 and 81.18±3.08 respectively), however substantial elevation (P<0.05) to 84.12±3.19 and 87.03±3.11 by days 120 and 180 has occurred. Finally, the percentage of spermatozoa with normal morphology increased significantly (P<0.05) from 68.07±2.58 at day 0 to 70.08±3.61, 75.14±4.29 and 85.31±3.63 at days 60, 120 and 180, respectively.

Table 1. Semen parameters of ejaculates recorded during primary evaluation (day 0) and during the treatment with Speman® (days 60, 120 and 180).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 0</th>
<th>Day 60</th>
<th>Day 120</th>
<th>Day 180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>4.55±0.41a</td>
<td>5.13±0.55b</td>
<td>6.18±0.47c</td>
<td>7.91±0.11a</td>
</tr>
<tr>
<td>Concentration (×10⁶/mL)</td>
<td>55.43±13.12a</td>
<td>61.81±15.21a</td>
<td>89.87±17.84b</td>
<td>118.12±20.12c</td>
</tr>
<tr>
<td>Total number of sperms per ejaculate (×10⁶/mL)</td>
<td>272.13±18.11a</td>
<td>297.14±17.14b</td>
<td>550.23±24.11c</td>
<td>913.34±23.08d</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>87.14±2.53a</td>
<td>89.15±1.89b</td>
<td>91.12±2.08c</td>
<td>92.11±2.13c</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>80.04±4.51a</td>
<td>81.18±3.08a</td>
<td>84.12±3.19b</td>
<td>87.03±3.11c</td>
</tr>
<tr>
<td>Spermatozoa with normal morphology (%)</td>
<td>68.07±2.58a</td>
<td>70.08±3.61b</td>
<td>75.14±4.29c</td>
<td>85.31±3.63a</td>
</tr>
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</table>

The values within a row marked with different superscripts differed at P<0.05.
DISCUSSION

Male dog infertility may be a very serious reason for huge financial losses in the canine breeding market (Ciribe et al., 2018). Many stud dogs have different reproductive problems that in many cases can be treated successfully if recognised in time (Schäfer-Somi, 2015). These are common challenges in small animal reproductive management and breeders usually seek accurate specific treatments to solve it.

Most often male dog infertility is a result of different prostatic and testicular diseases (Domosliawska et al., 2013), but in our study all oligospermic dogs were clinically healthy and the owners could not report any possible reason for the poor semen quality. Unfortunately, the reason for male infertility remains unknown in 70% of cases (Johnston et al., 2001).

Different supplements with a certain daily intake of micronutrients have been investigated for their ability to improve semen quality (Schäfer-Somi, 2015). Vitamins E and C have antioxidant effect, because they are able to pass cell membranes and to reduce free radical formation by lipid peroxidation inhibition (Suleiman et al., 1996). Both vitamins were successfully used in previous investigations to increase significantly the number of spermatozoa, enhance the motility and normal morphology percentage, and improve the total number of sperms per ejaculate in subfertile dogs (Hatamoto et al., 2006; Rocha et al., 2009; Lopes-Santiago et al., 2012; Kawakami et al., 2015). Similar results were reported in dogs with low fertility using selenium and vitamin E after one month (Domoslawksa et al., 2015) and in healthy dogs by long-term fish oil supplementation (Risso et al., 2016).

In our study, the results showed significant improvement in semen quality in dogs with oligozoospermia after daily treatment with Speman®. In a study with humans, Speman® had no consistent effect on mean seminal fluid volume, but a highly significant affect on sperm concentration, motility and in decreasing the morphologically abnormal sperms after 3-month treatment (Kadhem & Al-Ani, 2013). Analysing our results, we found that despite the decreased concentration and total number of spermatozoa per ejaculate, the percentage of their viability and total motility was within normal ranges. Most of the examined parameters, including semen volume, viability, total number of sperms per ejaculation and percentage of morphologically normal spermatozoa were significantly increased as early as the first control semen evaluation, which was performed on day 60 after the initial treatment, but were still out of fertile values and for that reason, the treatment was continued. It is acknowledged that the total duration of spermato genesis in the dog is approximately 62 days (Soares et al., 2009), which may serve as an evidence for the rapid effect of our treatment protocol. The concentration and total motility were also significantly improved, but after 120 days of treatment.

As the daily supplementation of selenium and vitamin E is very useful for semen quality in humans (Gaskins & Chavarro, 2018), their excessive use or excess dose can lead to sperm damage (Kumalic & Pinter, 2014). According to another investigation, any excess in vitamin E with folic acid can decrease the semen quality and affect adversely sperm motility in men (Danikowski et al., 2002). Kirchhoff et al. (2017) reported no effect on seminal parameters in healthy dogs supplemented with a high dose of sele-
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nium and vitamin E. The current study reported no adverse neither negative effects of Speman® treatment. In our opinion, the beneficial effects of Speman® in the treatment of dogs with oligozoospermia were due to the synergistic action of the various herbs included in its formulation, whose beneficial effect on the male reproductive organs have been reported. *Mucuna pruriens* reduces stress and improves the quality of semen, *Argyreia nervosa* is known for its aphrodisiac property, *Asteracantha longifolia* and *Hygrophi Glandularis* are reported to improve male sexual behaviour and reproductive function, *Tribulus terrestris* ameliorates the testicular development (Murcam et al., 2013).

In conclusion, the daily oral administration of Speman® for a period of 3 to 6 months is safe and very successful for improvement of oligozoospermia in dogs. Further research on fertility should be conducted in order to detect the real parameters of successful dog semen improvement after the treatment.

REFERENCES


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