



ZEUTERIN: ANALYSIS OF TESTOSTERONE LEVELS AND REPRODUCTIVE PARAMETERS AS COMPARED TO CONTROL GROUP – EVIDENCE FROM TWO YEAR CLINICAL STUDY

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INTRODUCTION

BACKGROUND: In the companion animal world, especially male dogs, there exists differing perspective towards the health benefits of testosterone on endocrine and metabolic functions. These benefits have been widely underrepresented as attempts to increase sterilization rates have focused on the potential promise of behavioral benefits associated with surgical castration, until now, the only method available to permanently sterilize male dogs, causing a reduction circulating testosterone 96-99%.

Testosterone, the male androgen, is essential for the development and maintenance of specific reproductive tissues as well as physiological functions such as increased muscle development, hair growth, joint development and bone mass. Accumulated evidence suggested that testosterone deficiency is associated with many diseases, such as cardiovascular disease in humans. Testosterone replacement therapy has been in pharmacology and clinical use since 1935. Today, oral and injectable testosterone therapy has been used widely in clinics. Recent research suggests that the negative impact neutering male dogs may have far reaching health implications for specific breeds.

OBJECTIVE: To determine the effects of Zeuterin (Zinc Gluconate Neutralized by Arginine), an FDA approved non-surgical method to sterilize male dogs 3 to 10 months of age, on testosterone levels of thirty (30) healthy male Beagle puppies, 6 months of age over a 24 month period as compared to a control group.

MATERIALS AND METHODS

Using a single breed-specific dataset, the objective was to examine the effect Zeuterin had on dogs followed for two years in a clinical setting. Forty-six-month-old beagles were placed into four groups of ten animals, each by random distribution according to testicular width. Each group received intratesticular injection treatments as follows:

- Group 1 – Bacteriostatic water (control);
- Group 2 – 25 mg zinc/arginine;
- Group 3 – 30 mg zinc/arginine; and
- Group 4 – 35 mg zinc/arginine.

This report provides relevant data from a study conducted as part of the FDA approval trial.

DATA COLLECTION:

The following data were collected during the course of the study.

Physiological Parameters and Clinical Observations

- Reproductive Parameters
- Progeny Tests
- Body Weight
- Rectal Temperature Reaction After Injection
- Ejaculatory Volume
- Serum Testosterone Level

Blood samples were collected at every three months by jugular venipuncture using Vacutainer brand evacuated blood collection tubes (Becton Dickinson and Company, Rutherford, New Jersey). The tube with EDTA additive was used for complete blood counts. The serum was separated from plasma by centrifuged at 2000 rpm for 30 minutes. The complete blood counts and serum chemistries were analyzed by Clinical Pathology Laboratory of the College of Veterinary Medicine, University of Missouri-Columbia, using an automated analytical system (Kodak Ektachem 500 H Analyzer, Rochester, NY). Serum testosterone was estimated by radioimmunoassay (RIA) (F.J. Auleta, B.V. Caldwell, and G.L. Hamilton. Androgens: Testosterone and Dihydrotestosterone. In: Methods of Hormone Radioimmunoassay, S.M. Jaffe and H.R. Behrman, eds., Academic Press, Inc., New York, pp. 359-370, 1974) using a Beckman LS-1800 Scintillation counter (Beckman Instruments, Inc., Fullerton, California).

RESULTS

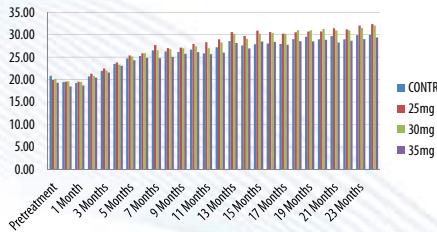
This study is the first report following the forty dogs for two years to collect the reproductive parameter, in which the evaluation of the testosterone is critical to the research. Blood testosterone levels were monitored by radioimmunoassay for two years. The ten control dogs achieved the normal canine value, group average ranging from 2.34 ng/mL – 3.69 ng/mL after a ten month sexual maturity period. The testosterone of the thirty (30) zinc/arginine treatment dogs group average ranged from 1.77 ng/mL – 2.17 ng/mL.

The results demonstrated that none of the treatments affected the dogs body's homeostatic functions. The body weight of all dogs increased; there was no clinical effect on rectal temperature; complete blood count and blood chemistry. At two years, blood testosterone levels for dogs in Groups 2-4 averaged 41-52% of the levels for dogs in Group 1. Relative to Group 1, dogs in Groups 2-4 had significantly reduced sizes of testes, epididymis, and prostate; histological examination revealed evidence of nonfunctional testes, and absence of sperm in the epididymis.

Progeny tests resulted in pregnancy for seven dogs in Group 1, and zero dogs in Groups 2-4. Semen was collected on a monthly basis for 21 months post – injection for dogs in Groups 2-4. No sperm were found in the ejaculate of 29 of these 30 dogs; one dog from Group 2 had sperm in the semen, but displayed severe oligospermia, low volume of ejaculation, as well as low sperm motility.

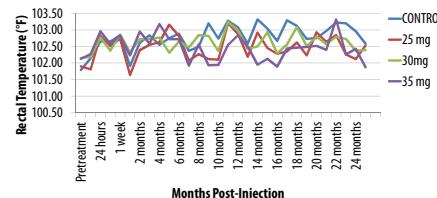
By the end of this dose titration study (twenty four months post-injection), the body weight of the control placebo group increased by 54.27% while body weight of the 25 mg, 30 mg, and 35 mg treated groups increased by 65.68%, 63.22%, and 58.96%, respectively.

Summary of Body Weights (lbs.)



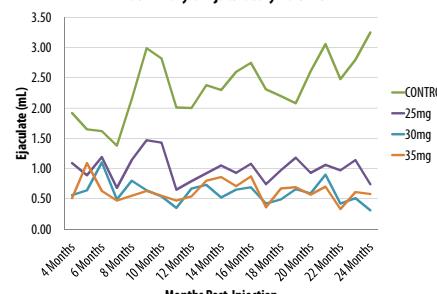
Rectal temperature varied from normal over time, and there was no clear pattern of increase or decrease. The variation in rectal temperature was most likely due to the excitement the dogs experienced when they were handled to measure rectal temperature.

Summary of Rectal Temperatures

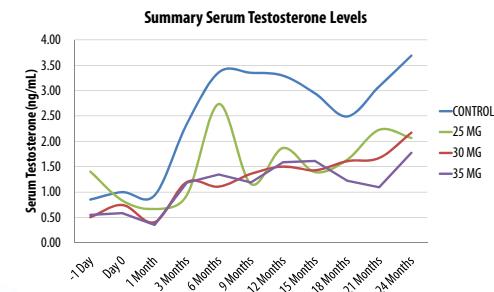


In the 25 mg treated group, one dog maintained severe oligospermia—the low concentration and low volume of this dog did not result in pregnancy when mated with a bitch in heat. In all semen collected from the 30 mg and 35 mg treated groups, no sperm were found and none of the dogs impregnated a bitch in heat.

Summary of Ejaculatory Volume



As compared to the control placebo group at the end of the study (twenty-four months post-injection), serum testosterone level of the 25 mg, 30 mg, and 35 mg treated groups had decreased by 44%, 41%, and 52% respectively. The treated animals were docile and easy to handle (not aggressive).



At the end of the study all vital organs were normal. Reproductive organs were all significantly reduced in size and weight including the prostate.

CONCLUSION

To our knowledge, this report is the first evidence which has studied male dogs 6 months of age to 30 months of age to analyze reproductive parameters utilizing a standard operating procedure. This is data has far reaching value for clinical and laboratory references focused on male dog reproductive research.

The percentage of testosterone that plays a role in sperm formation is not known. When spermatogenesis is stopped by intratesticular injection of zinc gluconate neutralized by arginine; the remaining testosterone is utilized for body development and maintenance. The results demonstrated that none of the treatments affected their body's homeostatic functions. The body weight of all dogs increased; there was no clinical effect on rectal temperature; complete blood count and blood chemistry. Testicular size of the dogs treated with Zeuterin was significantly reduced compared to the control group. When sperm production in testes is completely stopped, maintaining certain amount of testosterone is difficult. Therefore, more research is needed to understand the balance between testosterone levels and the development of secondary male characteristics such as muscle development.

Population management strategies play a significant role in animal overpopulation. The recent studies have shown increased risk of disease and cancer associated with spaying and neutering. Although more research needs to be conducted between the diseases or cancer and testosterone levels; it is not too early to pay attention to the facts of animal health.

Zeuterin was effective in causing permanent sterility in the treated animals while only reducing testosterone product 41-52%. These results have tremendous health implications for companion animals and service dogs.

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