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261 FERTILITY CONTROL BY GnRH ANALOGUES IN DOGS

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Abstract

GnRH plays a pivotal role in reproduction by stimulating the release of gonadotrophins. Chemical substitutions in the GnRH molecule lead to analogues possessing antagonist or agonist activity (Paramo RM *et al*. 1993 J. Reprod. Fertil. Suppl. 47, 387–397). The highly potent agonist analogue, Buserelin, with up to 20 times of potency, by increasing binding affinity, desensitizing competitive receptors, and resisting metabolic degradation, shuts down rather than stimulates reproductive function (Bertschinger HJ *et al*. 2001 J. Reprod. Fertil. Suppl. 57, 275–283). In man, Buserelin is employed in several gonadal hormone-dependent diseases and for prostatic cancers. We suppress gonadal function in male dogs using Buserelin. Eight intact male German sheep dogs 20 months old were divided into two groups; A, 4 subjects treated for pharmacological castration (Buserelin acetate, 0.3 mg/each, s.c., every 8 h for 30 days) (Suprefact-Aventis Pharma, Italy); B, 4 subjects treated with placebo (NaCl, 0.9%, s.c., every 8 h for 30 days). Plasma testosterone concentrations were measured twice a week by RIA using commercial kits (Coat-A-Count, Los Angeles, USA). Clinical examination of the male genital tract was conducted by ultrasound monitoring. Before and after the pharmacological treatment, semen was collected and evaluated for macroscopic and microscopic parameters. After treatment, testicular specimens were collected by orchiectomy, fixed in Bouin's solution, and embedded in paraffin wax. Thin sections were cut and stained with hematoxylin/eosin. The presence of germ cells (spermatogonia to spermatozoa, Sertoli and Leydig cells number) were analyzed. Randomly selected fields of transverse and longitudinal sections of seminiferous tubules were observed and analyzed using a computer assisted image analyzer (MONO system, Italy). The images acquired were segmented and binarized in order to obtain the masks of the tubular profiles; the mean values of the area, major and minor axes, mean diameter, and perimeter occupied by the testicular tubules were calculated automatically. Data were analyzed by ANOVA test. After Buserelin, all dogs (group A) showed a reduction in testicular and prostatic diameters compared to group B. Azoospermia was observed in group A. Histological examination revealed a statistically significant cell reduction of the germinal line (spermatogonia and spermatocytes, $P < 0.05$; spermatids and spermatozoa, $P < 0.001$). GnRH pharmacological treatment induced a cessation of normal spermatogenesis at the spermatocyte level while no statistical difference in morphometric parameters of seminiferous tubules were observed. The basic testosterone level (3.2 ± 1.3 ng/mL) rose to 12 ± 3.7 ng/mL (21° day) and than shut down to 0.5 ± 0.3 ng/mL (30° day), giving a long-term suppression. The present study demonstrates that Buserelin is an anti-fertility agent that gives suppression of reproductive function in male dogs. The method may have a clinical application. The utilization of a Buserelin depot will be a successive step.