ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
USE OF GnRH ANALOGUES, CALCIUM AND NALOXONE TO PRODUCE REVERSIBLE SUPPRESSION OF SPERMATOGENESIS IN THE DOG

M. Cinone, G. Aiudi, M. Albrizio, G.M. Lacalandra, G. De Vico*. Dep. Animal Production – Faculty of Veterinary Medicine, University of Bari
*Dep. Biological Sciences – Faculty of Biology, University of Naples. Str. prov. per Casamassima Km.3 – 70010 Valenzano (Bari) Italy. E-mail: m.cinone@veterinaria.uniba.it

Introduction - 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. The highly potent agonist analogue, Buserelin, with up to 20 times of potency, by increasing binding affinity, desensitizing competitive receptors, down-regulating GnRH receptors on pituitary gonadotropes and resisting metabolic degradation, shuts down rather than stimulates reproductive function (2,3). In humans, Buserelin is employed in several gonadal hormone-dependent diseases and for prostatic cancers. Recent studies evaluated Buserelin contraceptive effects with the aim to establish dose and length of the therapeutical treatment of prostatic carcinoma that leads to an inhibition of serum FSH, LH and testosterone (1). Histological evaluation of testicular sections obtained from buserelin treated rats evidenced characteristic changes such as decompensation of testicular compartments, progressive disorganization of seminiferous tubules, lack of spermatozoa in the lumen of tubules, loss of spermatogonial properties and of Sertoli cells. Moreover inflammatory, degenerative and necrotic areas associated with irreversible mineralization have been observed; in several cases these alterations were focal and associated to tubular calcification even if this is typical of the rat species (5). GnRH agonists have been proposed for the suppression of reproductive function in male dogs: males implanted with deslorelin long-acting implants show a decrease of serum testosterone to basal values. After removal of the implant, spermatogenesis reappears and fertility is restored (4).

Material and Methods - In this study, we combined the use of buserelin, a GnRH analogue, with calcium/naloxone association to obtain a desensitizing effect on LH receptors in an attempt to regulate spermatogenesis in dogs. Twenty post-pubertal mixed breed dogs were divided into two groups: (A) ten subjects were pharmacologically treated with a 30 day administration of buserelin acetate (Suprefact Aventis Pharma, Italy) 0.3 mg/each s.c. TID, associated with 0.1-mL/kg/bw i.m. injection SID of calcium gluconate 20% (New ICC, Upjohn, Italy) in which 0.4 mg/mL of naloxone hydrochloride (Diosynth, The Netherlands) were dissolved; (B) ten subjects received placebo (NaCl 0.9% s.c. TID for 30 days). At the end of the treatment, hemiorchidectomy was performed and 4 months later the second testis was removed. Clinical examination of the genital tract was done by ultrasound monitoring. Before and after pharmacological treatment and until total orchidectomy, semen was collected and evaluated for macroscopic and microscopic parameters. Plasma testosterone concentration was measured twice a week by EIA using commercial kits (Testosterone EIA Well, Radim, Italy). Testicular specimens were fixed in formalin solution, embedded in paraffin wax and stained with hematoxylin–eosin. The presence of germ cells (spermatogonia to spermatozoa, Sertoli and Leydig cells number) and the testicular tubule diameters were analyzed using a computer assisted image analyzer (Image-Pro Plus 6.2, Media Cybernetics, Italy) on randomly selected fields of transverse and longitudinal sections of seminiferous tubules. The images acquired were segmented and binarized in order to obtain the masks of the tubular profiles; 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 the ANOVA test.

**Results** - In the treated A group, all dogs showed a reduction in testicular and prostatic diameters compared to B group. Transient elevations in testosterone concentrations were observed in response to the early injections and reached baseline levels after 7±2 days. At the end of treatment, azoospermia and lower ejaculate volume was observed. At the histological observation, seminiferous tubules showed the block of spermatogenesis at the level of spermatocytes with a statistically significant cell reduction in spermatides and spermatozoa (P<0.001) whereas the seminiferous tubular morphometry was normal (Tab. 1). After 2 months from the end of the treatment, testosterone values had progressively regained normal values and, when measured immediately before total orchidectomy, sperm concentration and motility had returned to normal. Total orchidectomy demonstrated that a normal histological testicular structure had been restored. In the B group, no modifications were observed.

**Conclusions** - In this study, we demonstrate that GnRH analogue associated with calcium and naloxone induces suppression of the reproductive function in male dogs. The observed effect is reversible and doesn’t produce degenerative effects on testis histology. These devices designed for the shutdown of gonadal steroidogenesis and spermatogenesis would be useful for treating sex hormone-dependent syndromes such as malignant and benign prostatic tumors.

**References**


Tab. 1 - Image on the left represents seminiferous tubules of a GnRH treated dog analyzed by an automated image system while on the right a control subject is shown.