

**HISTOLOGY AND ELECTRONE MICROSCOPIC STUDY OF THE EFFECTS
OF AN GnRH AGONIST ON THE CANINE
TESTIS AND PROSTATE AFTER
3 MONTHS OF TREATMENT**

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Abstract

Using light and electrone microscopic techniques the structure of the testicle, epididymis and prostate gland of mature male dogs were studied 3 months after implantation with a slow release implant containing 6 mg GnRH agonist deslorelin. After 3 months of treatment, all tubules seminiferous showed atropic and aspermatogenic. Atrophy of the Leydig cells was observed in the interstitial tissue. The glandular epithelium of the prostate showed atrophy and was non secretory. At the electrone microscopic levels, Sertoli and Leydig cells showed a marked atrophy and their spermatogenic tubules were mostly lined with Sertoli cells at the basal lamina. The prostate gland showed atrophy of the nucleous and the epithelium was non secretory. The present data showed that a slow release implant containing GnRH agonist deslorelin can be used effectively to suppress fertility in male dogs.

Key words: Testicle, epididymis, prostste gland, Leydig cell, Sertoli cell, GnRH agonist, deslorelin

**PEMERIKSAAN HISTOLOGI DAN ELEKTRON MIKROSKOPIK
PADA TESTIS DAN PROSTATA ANJING SETELAH
DIBERI GnRH AGONIS SELAMA 3 BULAN**

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Abstrak

Telah dilakukan penelitian dengan menggunakan teknik mikroskop cahaya dan mikroskop elektron struktur dari testikel, epididimis dan glandula prostata anjing jantan dewasa yang diimplantasi selama 3 bulan dengan menggunakan pembebasan perlahan implan yang berisi 6 mg GnRH agonis deslorelin. Setelah implantasi selama 3 bulan, semua tubulus seminiferus menunjukkan atropi dan aspermatogenik. Atropi pada sel Leydig juga terobservasi pada jaringan interstitial. Glandula epithelium prostata menunjukkan atropi dan tidak bersekresi. Pada tingkatan mikroskop elektron, sel-sel Sertoli dan Leydig tampak dengan jelas atropi dan tubulus seminiferusnya hampir semuanya dibatasi oleh sel Sertoli pada dasar lamina. Glandula prostata menunjukkan atropi pada nukleus dan epiteliurnya tidak bersekresi. Data ini menunjukkan bahwa pembebasan perlahan implan yang mengandung GnRH agonis deslorelin dapat digunakan secara efektif untuk menekan fertilitas pada anjing jantan.

Kata kunci: Testikel, epididimis, glandula prostata, sel Leydig, sel Sertoli, GnRH agonist, deslorelin

1. Bagian Reproduksi & Kebidanan, FKH-UGM

Introduction

Microscopic studies on the testes and prostate of dogs after daily injection of 2.5 µg/kg GnRH agonist (buserelin) for 6 months have revealed involution of both glands (Sandow et al. 1980). (Vickery et al. 1984) reported that daily injection of 10 µg/kg/day of a potent GnRH agonist (D-Nal(2)6-LHRH) for 42 days in male dogs cause disruption of spermatogenesis, marked reduction of sperm number and atrophy of the epididymis and the prostate. A similar finding has been reported by (Dube et al. 1987). They found that the daily injection of 50 µg/kg of (D-Trp6) GnRH ethylamide in dogs for 4 months caused atrophy of the seminiferous tubules with the majority of tubules containing only composed of Sertoli cells and spermatogonia type A and B. However, little is known about the changes occurring in the testes and prostate of the male dogs after subcutaneous injection of a slow release implant containing the GnRH agonist deslorelin.

The aim of the present work was to study in detail the changes induced in the seminiferous tubules and the interstitial tissue in the dog testes and also the changes in the prostate at both the light and electron microscopic level after 3 months of implantation with a slow release implant containing the 6 mg deslorelin.

Materials and Methods

Animals. Eight male adult dogs ranging in age from 2 to 3 years and weighing between 15 and 30 kg were used in this study. They were assigned to 2 groups of 4 animals each. *Group 1*, (control) received blank implant and were sacrificed after 3 months of implantation. *Group 2*, received 6 mg/dog deslorelin implants and were sacrificed 3 months after implantation to assess the histology and electron microscopic structures of the testes, epididymis and the prostate of the dogs. All animals were housed one per cage and were fed Purina dog chow and tap water ad libitum. At the end of the experiment, they were euthanised using an overdose of phenobarbital administered intravenously.

GnRH agonist deslorelin implant. The GnRH agonist used in this experiment was deslorelin (D-Trp⁶-Pro⁹-des-Gly¹⁰-LHRH ethylamide). This agonist was prepared and supplied by Peptech Animal Health Pty Ltd, Sydney, Australia. The implant was formulated into bioimplants that were 0.23 X 15.2 mm and contained a 6 mg of deslorelin. The in vitro release rate of deslorelin was approximately 50 µg per 24 hours, as determined by HPLC and UV absorbance at 278 nm (Peptide Technology Limited, Sydney, Australia). Implants were prepackaged in 13 gauge needles and were injected subcutaneously in the neck between the shoulder blades under aseptic conditions.

Light microscopic studies. At sacrifice, the testes, epididymides and the prostate were removed, cleaned and fixed in Bouin's solution for 24 hours. The fixed tissues were then placed in 70% alcohol for 48 hours and processed for histology. Each specimen was embedded in paraffin wax and 5 µm sections were cut and stained with haematoxylin and eosin. Sections were examined under a light microscope (Olympus, Japan) at the magnification of (10 x, 20 x, 40 x, and 100 x). Photomicrographs of representative areas of the tissues were taken with a Leitz Orthoplan Microscope and Orthomat Camera (Germany) on Kodak (E100S) film.

Electron microscopic studies. Five small pieces of tissue were fixed in 3 % glutaraldehyde for 24 hours in 4 °C. The fixed specimens were sliced into 1 mm³ pieces and processed for routine transmission electron microscopy. The sliced tissues were washed several times in phosphate buffer and post-fixed in 1% Dalton's osmium tetroxide for one and a half hours at 4° C, then dehydrated and processed in ascending grades of ethanol as follows: ten changes in 70% ethanol for thirty minutes or until the solution was clear, two changes in 90 % ethanol over 10 minutes, 5 minutes in 95 %, three changes in absolute alcohol for 5 minutes

and two changes in propylene oxide and epon araldite mixture (60 : 40) at 4° C for one hour. The tissue samples were then soaked overnight in pure epon on a rotator. All pieces of tissues were embedded in freshly prepared epoxy resin in embedding capsules and allowed to polymerise for 24 hours at 60° C. Block surfaces were trimmed with a hacksaw and single-edge razor blades. Using glass knives and a Reschert OMU3 ultramicrotome, one micron, semi-thin sections were cut and stained with toluidine blue. These sections were examined with a light microscope (Olympus BH2) for tissue orientation, after which ultrathin sections of about 70 to 90 nm thickness were cut using a diamond or glass knife on the ultramicrotome. The ultrathin sections were chloroformed and collected on 200 mesh copper grids and allowed to dry on filter paper. These sections were stained with aqueous uranyl acetate for 7 minutes, washed 40 - 60 times in double distilled water and allowed to dry on filter paper. These sections were counterstained with freshly prepared lead citrate for 7 minutes, washed 40 - 60 times in double distilled water and dried on filter paper. The grids were carbon coated (Dynavac). Ultrathin sections were examined using Philip's 301 and or CM100 Biotwin electron microscopes operated at an accelerating voltage of 80kV.

Results

Light microscope observations

In the control dog, there was active spermatogenesis in the seminiferous tubules

(Fig. 1a). The ductus epididymides are characterised by the height of the pseudostratified epithelium with microvilli, spermatozoa were observed in the ductus epididymides. The prostate exhibits characteristic acini lined with simple columnar epithelium surrounded by thin septae of connective tissue.

In the treated dogs, atrophic and aspermatogenic seminiferous tubules occurred throughout the section (Fig.1b). Only Sertoli cells and a few spermatogonia were present in the tubules. Severe epithelial atrophy, vacuolation and loss of epithelial cilia were observed in the ductus epididymides. No spermatozoa were observed in the ductus epididymides. The prostate showed complete atrophy of the glandular epithelium and a relative increase of the unchanged connective tissue.

Ultrastructural findings

In the dogs treated with deslorelin for 3 months, the nucleus of Sertoli cells appears smaller and more spherical, and the cell is definitely smaller than in the control dogs (Fig. 2b and 2a). The spermatogonia appeared normal (Fig. 2b).

In the Leydig cells of the control dog (Fig. 3a), nuclei were generally round and heterochromatin occupied a thin ring, lining the nuclear envelope. In contrast, in the Leydig cells of deslorelin treated dog after 3 months of implantation with 6 mg deslorelin nuclei were atrophied (Fig. 3b).

The glandular epithelium of the prostate of the dogs treated with 6mg deslorelin revealed reduction of epithelial height, atrophy of the nucleus and no secretory granules (Fig. 4b), whereas there were no changes in the control dogs (Fig. 4a).

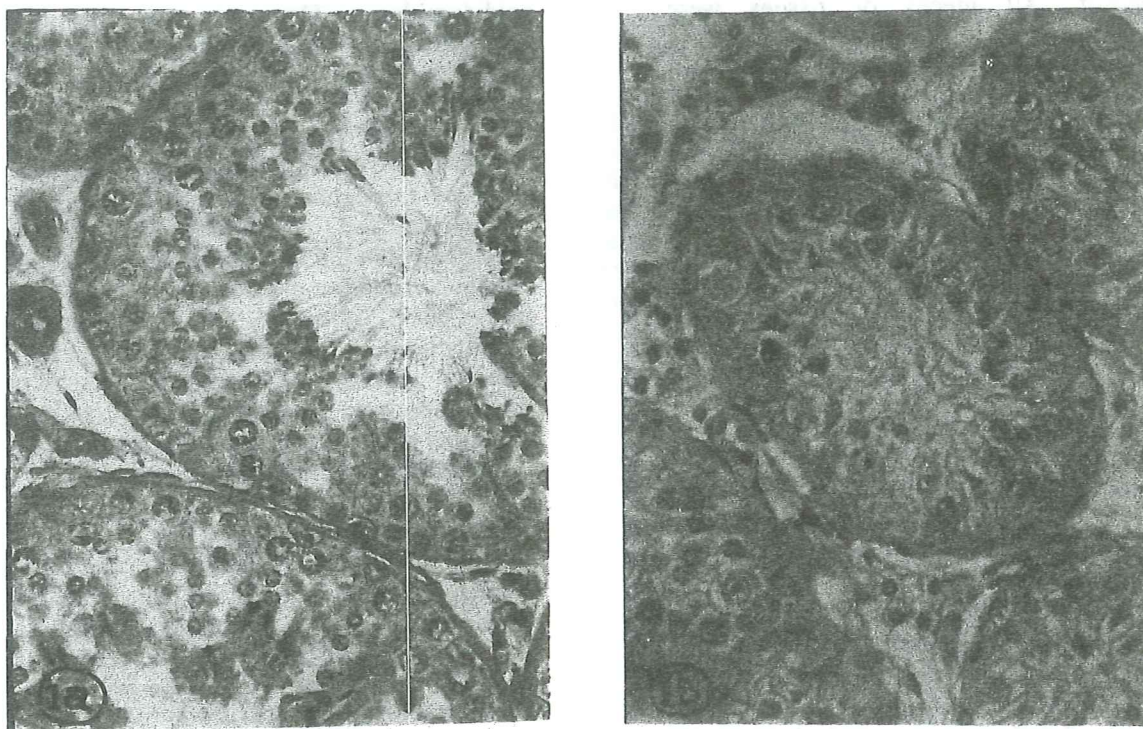


Fig.1(a) Light micrographs of seminiferous tubules in **(a)**. control dog. Notice active spermatogenesis in the seminiferous tubules. H.E.S x 100. **(b)**. dog treated with deslorelin for 3 months. Notice atrophic and aspermatogenic seminiferous tubules and atrophy of the Leydig cells in the interstitial tissue. H.E.S x 128

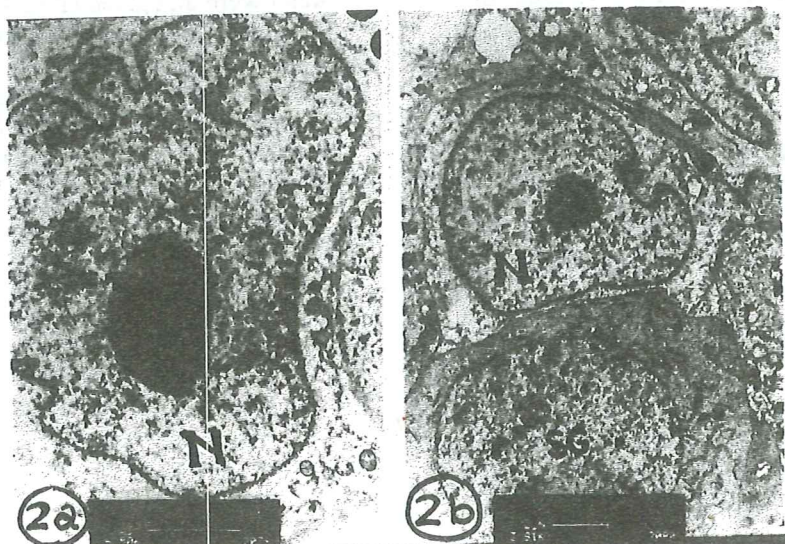


Fig. 2. Electron microscopic view of a Sertoli cell in **(a)**. control dog. Note that the nucleus (N) is irregular with the typical nucleolus. The bar represents 2 μ m. **(b)**. dog treated with deslorelin for 3 months. Note atrophy of the nucleus(N). The spermatogonia (SG) appear normal. The bar represents 2 μ m.

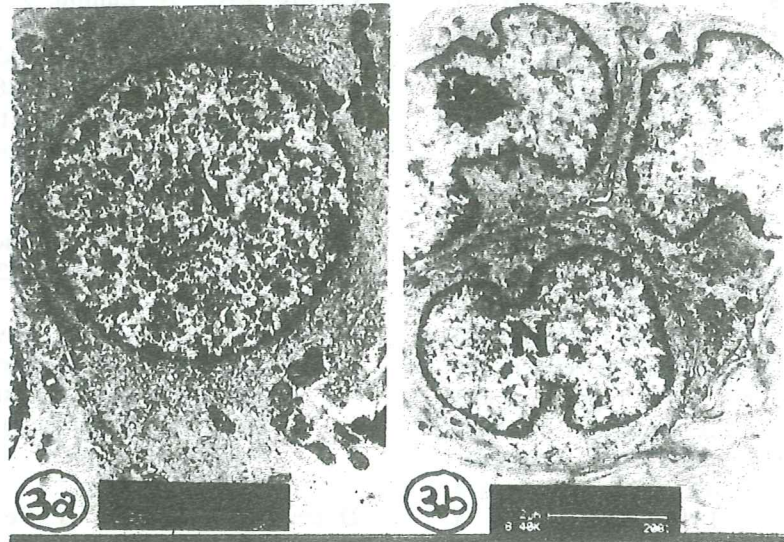


Fig. 3. Electron microscopic view of a Leydig cell in (a). control dog. The nucleus is round (N) and shows finely granulated heterochromatin. The bar represents 2 µm. (b), dog treated with deslorelin for 3 months. Note atrophy of the nucleus (N). The bar represents 2 µm.

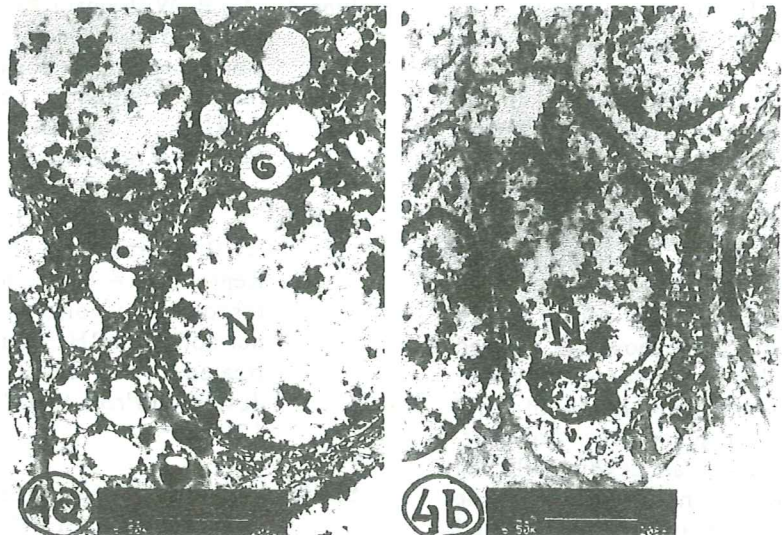


Fig. 4. Electron microscopic view of a glandular epithelium of the prostate gland in (a). control dog. Note tall columnar epithelium, nucleus (N) and secretory granule (G). The bar represents 2 µm. (b), dog treated with deslorelin for 3 months. Note that the nucleus (N) is shrunken and has no secretory granules. The bar represents 2 µm.

Discussion

After 3 months of implantation with 6 mg deslorelin all dogs showed atrophic and aspermatogenic seminiferous tubules. Atrophy of the Leydig cells was also observed in the interstitial tissue. The glandular epithelium of the prostate showed atrophy and was non secretory. Similar histological findings were described by (Cavitte *et al.* 1988) in dogs after 91 days of treatment with 50 µg/GnRH agonist (D-Trp⁶ LHRH) /kg. The lack of germ cells in the lumen of seminiferous tubules and the apparent increase in Sertoli cell number is indicative of tubule atrophy (Dube *et al.* 1987).

Chronic stimulation of gonadotropes with a GnRH agonist causes pituitary desensitization with a complete loss of bioactive LH (St-Arnaud R *et al.* 1986). Junaidi *et al.* (1998) found that the long term effect of the GnRH agonist deslorelin in the male dog showed full down regulation and desensitisation of the anterior pituitary gland, and explains the loss of testicular function. At the testicular level, the absence of stimulation by LH would cause a reduction in testosterone secretion which can explain Leydig cell atrophy and would be secondarily responsible for the atrophy of the seminiferous tubules deprived of androgens. These result are in agreement with previous study in the dogs, that show that GnRH agonist deslorelin will dropped testosterone and LH concentrations to zero started at 25 days after implantation (Junaidi *et al.* 1997).

At the electron microscope level in the dogs 3 months after implantation, Sertoli cells showed a marked atrophy. Their spermatogenic tubules were mostly lined with Sertoli cells at the basal lamina. Similar findings were described by Dube *et al.*, (1987) in dogs treated with daily injection of 50 µg of (D-Trp⁶)GnRH ethylamide for 16 weeks. Most Leydig cells showed a marked atrophy with smaller nuclei containing more heterochromatin and accumulation of lipid droplets. This image is, however, heterogeneous, since not all Leydig cells had the same appearance thus confirming the nation of heterogeneity in Leydig cell function (Christensen, 1975). In the prostate of dogs 3 months after implantation atrophy

of the nucleus was severe and the epithelium was non secretory.

The present data clearly shows that a slow release implant containing the 6 mg GnRH agonist deslorelin resulted suppression of spermatogenesis in male dogs.

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