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# PERIPHERAL NEUROPATHY IN EXPERIMENTAL BORNA DISEASE VIRUS INFECTION IN LEWIS RATS

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Borna disease virus (BDV) infection, naturally occurring in horses and sheep in Germany and Switzerland (6, 2) causes a severe non purulent meningoencephalitis and retinitis in adult Lewis rats (3, 4). The histopathological lesions are not induced by the virus itself, but by a virus-specific CD 4+ T-cell-mediated immunopathologic mechanism presumably of delayed hypersensitivity type (4, 1).

The virus seems to be of high biological variability (5). Depending on the virus preparation used, rats develop varying clinical disease, including fatal neurological disorder, obesity syndrome with infertility and emaciation.

Here we report on the occurrence of spinal cord and peripheral nerve lesions in experimental Borna disease.

4 weeks old Lewis rats were inoculated intracerebrally with BDV and sacrificed at varying intervals from day 9 up to day 99 after inoculation (p.i.). Brain, spinal cord and sciatic nerves were processed for histopathology, immunocytochemistry and electronmicroscopy. In addition sciatic nerves were processed by the teased fiber technique.

Clinically, beginning with day 19 p. i., the animals developed motoric disturbances including spastic paresis and muscular atrophy of the hindlegs.

Histologically, first encephalitic lesions were observed 14 days p.i. Spinal cord lesions in the lumbar segments L 4 – L 6, the origin of

the sciatic nerve, were found by day 17 p.i. Similar as in the brain spinal cord alterations consisted of mononuclear perivascular and parenchymal infiltrates mainly in the gray matter, followed by marked astrogliosis in the later stages of disease. Overt necrosis of neuronal cell bodies or neuronophagia could not be found. However, occasionally slight chromatolytic changes were seen in dorsal and ventral horn neurons.

In the sciatic nerves and corresponding nerve roots first changes were seen 17 days p.i., resulting in a progressive combined axon and myelin degeneration of Wallerian type. Compared to the severe encephalitic lesions, in the peripheral nerves only a moderate inflammatory response developed, consisting predominantly of macrophage-like cells.

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# NOTE 2: GENITAL LESIONS AND EVALUATION OF UTERINE NUCLEAR ESTROGEN RECEPTORS (ER)

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Several experiments have been carried out in order to study the effect of  $\beta$ -agonists on performance of pigs, most of them with cimaterol and few with clenbuterol (Fiems, 1987; Hanrahan 1987). Although particular attention was always given to the performance, carcass and meat quality at present no data are available about genital pathology following  $\beta$ -agonists administration. Therefore the aim of this work is to point out the involvement of female genital tract following the use of  $\beta$ -agonists in finishing pigs.

The number of examined animals and doses of clenbuterol employed for the experiment are described in «Note 1». Ovaries and uteri were weighed and samples from all genital organs were examined histologically after fixation in 10% buffered formalin, samples from uteri were also frozen and examined for nuclear estrogen receptors (ER) with a commercially available monoclonal antibody (ER-ICA, Abbot laboratories, Chicago) using an immunohistochemical technique.

The ovaries of controls showed normal cycling activity as follicle maturation and presence of corpora lutea. In detail, following the classification of Leizer et al. (1988), the ovaries of subject nr. 5 were in metestrus, nr. 6 in middle diestrus, nr. 7 proestrus, nr. 8 in middle diestrus (Tab. 1). By contrast, the ovaries of all treated animals showed the same macroscopical and histological features. The main

finding (tab. 1) was represented by the significant difference of weight between treated (average of 3.44 g) and controls (6.62 g). No corpora lutea were present while a microcystic degeneration was evident, being the biggest follicles about 5 mm wide, resembling in some way the pre-puberal immature ovaries.

The uteri of control animals had an average weight of 765.75 g and normal morphology related to the ovarian activity. Test for the presence of ER was positive in all uteri at a medium intensity.

The uteri of treated animals had an average weight of 264.5 g. The volume was significantly decreased, thereby the uterine wall was thinner than in controls. Histologically the mucosa showed inactivity and the number of endometrial glands was lower than in controls, the glandular epithelium was low with an elongated nucleus occupying almost the whole cytoplasm as well as the epithelium of the endometrial surface.

The immunoperoxidase reaction for ER was strongly positive in all uteri, except for uterus nr. 3 which was completely negative because of a possible artefact.

In cervix and vagina of controls, thickness of the epithelium was variable, depending on the stage of the estrous cycle, up to 10–20 cell layers.

In all treated animals there were only few cell layers (two to four) as in the luteal stage of the normal sexual cycle, but in this case there was complete absence of corpora lutea.

In conclusion the most important findings are represented by the ovarian inactivity associated to the atrophy, or a delayed development of the rest of the genital tract. This hypothesis is supported also by the significant difference between ovarian and uterine weight in treated and control pigs and particularly by the histological findings of the endometrial, cervical and vaginal epithelial structures. The detection of ER was slightly prevalent in treated animals and was consistent with the quantitative investigations described in «Note 1». Although the number of subjects employed in the experiment needs to be increased to better understand and confirm the detected lesions, we assume that they may represent the consequence of long time administration of  $\beta$ -agonists to fattening pigs, which may influence the metabolism of some cell populations of the genital tract, like for example the granulosa cells which are provided of membrane receptors for  $\beta$ -agonists (Hsueh et al. 1984).

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### ULTRASTRUCTURAL MODIFICATIONS OF LAMB TESTICLE SPERMATIDS INDUCED BY OESTRADIOL AND TRENBOLONE ACETATE

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Oestradiol and trenbolone acetate are commonly used as anabolics in order to improve the yield of stock animals, as well as the appearance and quality of their meat. According to Michel and Beaulieu (1983), these substances act in a direct fashion via specific receptors.

The continuous use of hormones may induce carcinogenesis (Hoffman, 1986). On the other hand, Rao and Chinoy (1984) and Bellido and Gaytán (1985) found the administration of oestrogens in male rats to induce marked testicle atrophy.

### Material and methods

The study was carried out on 16 thirty-day-old lambs, in 12 of which pellets were implanted subcutaneously containing 2.5 mg oestradiol and 17.5 mg trenbolone acetate. The other 4 lambs were used as controls (see Table 1).

### Results

Lot I: Of the various cells occurring in the seminiferous duct, spermatids are worth mentioning on account of their atypical morphological features. They occurred in small numbers and were located in the central zone of the seminiferous ducts, related by thin peduncles to Sertoli cells; they were spherical to elliptical in shape,

Tab. 1 – summary of results

pig n.	weight of ovary (g)		uterus (g)	pig (kg)	ER-ICA	stage of sexual cycle
	right	left				
TREATED						
1	2.47	2.59	308	140	+	—
2	3.66	3.51	267	127.5	+	—
3	4.32	3.97	187	136	—	—
4	3.30	3.70	280	126	++	—
average weight	3.44	264.5	132.4			
CONTROLS						
5	3.86	4.33	661	134±		metestrus
6	6.77	16.16	583	141—		middle diestrus
7	6	5.72	851	172±		proestrus
8	4.11	6.44	932	165±		middle diestrus
average weight	6.62	765.75	153			

and the electron density of their hyaloplasm and nucleus was quite low. Their nucleus was spherical, with outstanding nucleolus.

Cytoplasmic organellae occurred in small numbers and their mitochondria had elongated shapes. The chromatoid body was quite prominent, with clear signs of fragmentation.

Lot II: Spermatids were more abundant in this group. They were elongated in shape and occasionally binucleate, with outstanding nucleoli.

The cytoplasm was clearly seen and the granular endoplasmic reticulum (GER) and particularly the Golgi complex were poorly developed. Like in Group I, mitochondria were elongated in shape; the chromatoid body, fragmented and occasionally related to non-specific vesicles, lay prominently at one of the cell poles, close to the nucleus.

Control group: Neither group showed this type of altered spermatids.

### Discussion

The spermatid cells found in the experimental animals were different from those of the control animals in their undifferentiation and even in their binucleate nature, which is indicative of a dysfunction of the seminiferous epithelium, consistent with earlier findings by Wiggins et al. (1980). Occasionally, the experimental animals also showed no connection between their spermatids and Sertoli cells.