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Artikel: Clenbuterol long-term administration in finishing female pigs : note 1 : effects on oestrogen and progesterone receptor (ER and PgR) distribution and concentration in genital tract

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tized host could exist. Different types of glomerulonephritis characterized by IgG, IgM and C_3 deposition have been already described in dogs and often associated to different pathological conditions.
This is the first description of an IgA nephropathy in a dog affected by a chronic parasitic disease. Although the morphological, electromicroscopical and immunological patterns are strongly resembling IgA nephropathy other attempts need to discriminate this GN from other immunological disturbances. IgA deposits in fact have been also found in proliferative lupus GN and other proliferative GN (Jennette, 1988) but the immunohistological demonstration of predominantly mesangial IgA immunostaining can be accepted as criteria for making a pathological diagnosis of IgA nephropathy.

Literature

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CLENBUTEROL LONG-TERM ADMINISTRATION IN FINISHING FEMALE PIGS. NOTE 1: EFFECTS ON OESTROGEN AND PROGESTERONE RECEPTOR (ER AND PgR) DISTRIBUTION AND CONCENTRATION IN GENITAL TRACT

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Clenbuterol is a bronchodilator and tocolytic agent widely used in veterinary medicine. When this ß2-agonist is chronically administered, either orally or parentally, fat deposition is reduced (up to 30%) and protein accretion occurs (1). Therefore clenbuterol is illegally employed as «repartitioning agent» in animal husbandry, since it powerfully stimulates muscle growth in ruminants, pigs and poultry (2). Then, we noted that heifers longterm administered with clenbuterol at high dosages (our data not yet published), presented changes in the genital tract, consisting in an abnormal collection of mucus in uterus and vagina associated with ovarian alterations. Furthermore, ER and PgR progesterone receptor concentrations, investigated in different areas of the uterus, revealed a noteworthy increase. We have obtained similar results in female rats (3). Aim of our experiment was to verify if clenbuterol is able to induce analogous alterations in the reproductive system of female pigs.

Material and methods

Eight Landrace x Large white female pigs, weighing about 120 kg, were randomly assigned to each of two experimental pelleted diets, containing 0 and 1 ppm of clenbuterol respectively. Animals were housed in two pens with slatted floor, in which they were exposed to existing photoperiod and ambient temperature. Feed and water were provided ad libitum. On the 48th day of treatment pigs were killed by exsanguination after a light electric shock. Blood samples were collected after 0, 24, and 48 days of treatment, in order to evaluate estrogen and progesterone serum levels, using RIA kits (Double Antibody Estradiol° and Coat-A-Count° DPC, USA). ER and PgR levels were measured by a modified DCC method on tissue samples (1 g of mucusa) collected from five different areas of the genital tract: vagina (A), cervix (B), body (C), first (D) and second (E) half of uterine horns. Steroid receptor concentrations, reported in Tab. 1 and Tab. 2, are expressed as femoles of bound hormone/mg of cytosol protein. Data obtained were statistically processed using the analysis of variance (two way with replication) and Duncan's test.

Results and discussion

As reported in Tab. 2, the results revealed that clenbuterol affected both ER and PgR concentrations causing significant increases in treated animals ($p < 0.05$). The distribution of steroid receptors ranges from the lower values found in the vagina and in the cervix to the higher ones found in the uterus, mainly in the horns of either two groups. Besides, clenbuterol treatment never affected ER and PgR distribution along the genital tract (Tab. 1 and Tab. 2). Estradiol and progesterone serum levels, in accordance with macroscopical and histological observations, suggested a lack in ovarian activity in treated pigs. By contrast, in the control group, two subjects were in luteal phase, one in follicular phase and the remaining one in metestrus (Tab. 3).

Tab. 1: Effects of clenbuterol treatment on ER and PgR distribution and concentration in porcine genital tract (mean values ± ES)

	TREATED		CONTROLS	
	ER	PgR	ER	PgR
A) Vagina	54± 23	96± 42	—	94 ± 22
B) Cervix	79± 33	369± 162	12± 0.6	117 ± 35
C) Body	268± 114	560± 168	25± 6	214± 885
D) Horn (1st half)	304± 89	828± 176	41± 6	290± 120
E) Horn (2nd half)	227± 81	558± 44	55± 3	388± 98

Tab. 2: Statistical evaluation of results

	Treatment (T)		Samples (S)					Interact	
	control	clen	A	B	C	D	E	F values (T)	(S)
ER	27a	186b	27a	46a	146b	172b	141b	20.92**	2.82* 1.55
PgR	221a	482b	95a	243a	387b	559b	473b	14.01**	5.59** 1.63

*P<0.05; **P<0.01; a,b P<0.05.

Tab. 3: Oestradiol and Progesterone serum levels

Day	Treated					Controls			
0	E_2	15	95	31	12	12	15	17	10
	Pg	0.1	0.3	0.1	0.1	0.1	0.1	1.4	12.5
24	E_2	100	30	14	9	31	18	10	15
	Pg	1	0.6	1.6	0.2	29	18	28	40
48	E_2	7	33	5	11	11	31	15	37
	Pg	0.1	0.1	0.2	0.3	25	12	29	0.3

E_2 = pg/ml Pg = ng/ml

In summary, clenbuterol long-term administration significantly affects uterine ER and PgR concentrations in female pigs, as well as we have found in previous experiments carried out in female rats (3) and in cows (unpublished data). Furthermore, the modifications observed in treated animals, are related with estradiol and progesterone serum levels. Data obtained suggest that clenbuterol may cause changes, producing a lack in ovarian activity, confirmed by the histological findings reported in the Note 2. The mechanism by which clenbuterol exerts this action is not clear, but we may suppose two contrasting ways: a direct effect on steroid receptors or an indirect effect upon the ovaries mediated via the hypothalamic-hypophyseal axis.

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ULTRASTRUCTURAL AND PHYSIOPATHOLOGICAL GLOMERULAR FILTRATION BARRIER AFTER EXPERIMENTAL ENDOTOXIN SHOCK

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The aim of the present work was to evaluate the relationship between physiopathological parameters (plasma proteins and urine test) and renal histopathology particularly structural architecture of the glomerular filtration barrier, in experimental endotoxin shock.

Material and methods

In the present work a histopathological study is carried out on the glomerular filtration barrier in 20 Large-white pigs weighing 20 kg, subjected to experimental intravenous inoculations of endotoxin from *Salmonella enteritidis.*

The study is completed with the determination of plasma protein levels and with urine tests: determination of pH, density, proteins, glucose, ketone body levels and urinary sediment.

Table 1. Procedure of the experiment

Group	Experimental Animals	Control	Shock Time	Doses
I	4	I	10 days	2
II	4	I	20 days	4
III	4	I	30 days	6
IV	4	I	50 days	8

Dose 0.125 mg of *S.enteritidis* endotoxin per Kg body weight.

Results and discussion

Histopathological findings: The glomerular basement membrane showed an increase in its diameter parallel to the number of doses inoculated and the time of shock; an increase which always coincided with the degree of structural disorganization (Redondo et al., 1987).

Physiopathological findings: The effective and selective glomerular filtration barrier to diffusion is probably the basement membrane. The permeability of the basement membrane may be altered in shock processes in which high molecular compounds (immune complexes) are deposited on or within the barrier. Proteinuria may result from a decreased capacity for tubular resorption or tubular damage, but mostly proteinuria is a consequence of altered glomerular filtration. Decrease in urinary output characteristic of prolonged septic shock processes, is closely connected with the increase of protein catabolism and proteinuria (Shirota et al., 1983).

Furthermore, the density of the urine is higher in the last groups, which correlates with the tubular disintegration detected and the presence of calcium oxalate crystals (Rosenbruck et al., 1984; Redondo et al., 1987). Likewise, this increase of density is characteristic of prolonged shock processes.

Table 2. Urine and blood test

Urine Test

	Control	Group I	Group II	Group III	Group IV
Transparency	bright	turbid+	trubid+	turbid+	turbid+
pH	6.2±0.2	5.2±0.3	5.3±0.1	5.1±0.3	4.9±0.2
Density	1.015±0.02	1.015±0.1	1.020±0.01	1.030±0.02	1.035±0.02
Proteins	–	–	5.4±0.3 g/dl	6.3±0.4 g/dl	7.9±0.2 g/dl
Glucose	–	–	–	–	–
Ketone bodies	–	–	–	–	–
Sediment	–	–	–	–	–
Erythrocytes	–	–	–	–	–
Leukocytes	–	–	–	–	–
Epithelial Cells	–	+	++	+++	+++
Crystals	–	calcic oxalate+	calcic oxalate+	calcic oxalate++	calcic oxalate++
Cylinders	–				
– Hyaline		+++	+++	+	+
– Epithelial		+++	+++	+	+
– Cereous		+	+	+++	+++