Veterinary Research Communications, 30(Suppl. 1) (2006) 365–367
DOI: 10.1007/s11259-006-0082-0

Immunohistochemical Evaluation of Estrogen Receptor β Expression in Calf Prostates with Squamous Metaplasia

R. Stocchi*, G. Renzoni, S. Rea, S. Cecchini and A.R. Loschi
Department of Veterinary Science, Section of Animal Pathology, Prophylaxis and Food Hygiene, Faculty of Veterinary Medicine, University of Camerino, 62024 Matelica (MC), Italy
**Correspondence: E-mail: roberta.stocchi@unicam.it*

Stocchi, R., Renzoni, G., Rea, S., Cecchini, S. and Loschi, A.R., 2006. Immunohistochemical evaluation of estrogen receptor β expression in calf prostates with squamous metaplasia. *Veterinary Research Communications*, **30(Suppl. 1)**, 365–367

Keywords: anabolic substances, calves, estrogen receptor β, immunohistochemistry, prostate

Abbreviations: ERα, estrogen receptor α; ERβ, estrogen receptor β; IHC, immunohistochemical; BSA, bovine serum albumin

INTRODUCTION

Repeated administration of substances belonging to the group of anabolic drugs, especially those with an estrogenic effect, such as diethylstilbestrol (DES) employed for zootechnical purposes, has long been known to cause morphological modifications of the accessory sex gland epithelium. Long-lasting estrogenic action can also be detected through histological screening of slaughtered animals. In the sexual glands of male calves experimentally treated with estrogens these modifications are chiefly related to proliferation and metaplastic changes of the basal cells, as previously shown by immunohistochemistry using antibodies against cytokeratins 13, 15 and 16 (Finazzi *et al.*, 1994; Groot *et al.*, 2000). In comparative medicine, interesting researches have recently been carried out on transgenic mice used as an experimental model to study the etiopathogenetic mechanism of benign prostate hyperplasia and prostatic carcinoma (Jarred *et al.*, 2002; Pearce and Jordan, 2004), two of the most frequent prostate pathologies of human beings. According to these studies, it seems that when hyperplastic, metaplastic and neoplastic changes take place under hormonal stimulation a key role is played by the complex interaction between the different hormonal substances and their epithelial receptors. In particular, the estrogen activity in the prostate appears to be regulated by two different kinds of receptors: ERα, which can be found mainly in the stromal cells, and ERβ, mainly located in the epithelium (Weihua *et al.*, 2002).

The aim of this preliminary report is to evaluate the expression of ERβ receptors in the prostates of regularly slaughtered calves suspected of treatment with estrogenic hormonal substances on the basis of hyperplastic and/or metaplastic lesions of the prostatic epithelium.

MATERIALS AND METHODS

The disseminated prostates of eleven regularly slaughtered calves, aged 7–8 months, were examined. These samples had previously been subjected to traditional histological examination and IHC screening using anti-cytokeratin 13, 15 and 16 monoclonal antibodies (Stocchi *et al.*, 2004) to check for the mentioned above morpho-immunopathological changes. One of these samples was negative using both screening methods and therefore was used as negative control. The other 10 were positive and showed either diffuse or focal squamous metaplasia, in some cases associated with cystic ectasia and hypersecretion: specifically, 6 specimens showed diffuse squamous metaplasia already visible upon traditional histological examination and the other 4 samples were focally positive for mild hyperplasia and metaplasia only using the IHC technique.

Three μm thick histological slices were analysed using the IHC technique, according to the avidin-biotin-peroxidase method (ABC, Vector Lab., Burlingame, USA). The sections were incubated at first with 3% hydrogen peroxide in methanol, then with 10% normal goat serum and were finally microwave heated (750 W) in 10 mM citrate buffer for 2 cycles of 8 min each. Afterwards, the sections were incubated at 4°C overnight with primary rabbit polyclonal PA1-313 anti-ERβ antibodies (ABR, Golden, Co), in 1% BSA (Sigma, Saint Louis, USA). The samples were then incubated for 30 min with secondary goat anti-rabbit antibody (Vector Lab., Burlingame, USA) and diluted 1:200 in BSA. In some sections the primary antibody was replaced with normal goat serum at the same concentration. Washings were performed using TBS buffer at pH 7.3 and 3% 3,3′-diaminobenzidine was used as chromogen. After light nuclear counterstaining with Mayer's haematoxylin, sections were passed through a progressive series of alcohol-xylene, mounted with EUKIT resin and then observed under an Olympus BX50 light microscope. Cells showing rust-brown nuclear and/or cytoplasmic precipitates were considered positive.

RESULTS

The IHC examination using anti-ERβ antibodies showed the presence of positive epithelial cells in all samples. The positive immunoreaction was revealed by the intense brown colour of nuclei and/or the presence of fine rust-brown granular precipitates in the cytoplasm. In the acini made up of secretory epithelium, the immunoreactivity was variable, being present either in the nucleus or in the cytoplasm or in both locations. In the most peripheral acini, mainly made up of mucous cells, only nuclear positivity was shown, with rare exceptions.

Taking into consideration the distribution of receptor expression, the most diffuse positivity was observed in the sample assumed as negative control because of the immunohistochemically ascertained absence of basal cell hyperplasia. In the other samples, the number of immunopositive cells decreased with the more diffuse and intense hyperplastic/metaplastic phenomena. This was well documented at higher magnifications, which clearly showed the lack of immunopositivity in all the cells making up both the hyperplastic areas - characterised by the presence of papillary buds of multilayered epithelium invading the acini - and even the small clusters of cells affected by squamous metaplasia.

Finally, the urethral epithelium, the ductal epithelium and the stroma cells were negative in all samples.

DISCUSSION

The IHC results show that it is possible to apply this technique to the bovine species. Of even greater importance is the opportunity to compare this study with the previous one carried out on the same samples of prostatic tissue to identify the proliferating cellular types. According to other authors (Finazzi *et al.*, 1994; Groot *et al.*, 2000), this previous investigation showed that the proliferating and metaplastic cells belong to the basal compartment, on the basis of the kind of cytokeratins expressed. Contrary to the "normal" cells, basal cells were non-expressive of ERβ receptors. This would suggest that the ERα receptors could play a fundamental role in causing the onset of the mentioned above changes due to alteration of the ERα/ERβ balance induced by the use of estrogens, especially synthetic ones.

Therefore, this research needs to be continued particularly from the point of view of meat inspection in order to define useful diagnostic tool for slaughtered animals. This is a very topical issue because the use of hormonal anabolic substances is still widespread and is even increasing, especially in calf breeding, as shown by the non-conformities detected during the monitoring activity provided by the National Residue Plan 2004.

REFERENCES

Finazzi, M., Gelmetti, D., Grieco, V. and Bontempi, W., 1994. Reperti istologici ed immunoistochimici delle ghiandole annesse all'apparato genitale in vitelli trattati con promotori di crescita. *Argomenti di Patologia Veterinaria*, **36**, 273–283

Groot, M.J., Ossenkoppele, J.S. and Bakker, R., 2000. Changes in cytokeratin expression in accessori sex glands of growth promoter treated veal calves: influences of treatment schedule ond combination of drugs. *European Journal of Veterinary Pathology*, **6**, 19–24

Jarred, R.A., McPherson, S.J., Bianco, J.J., Couse, J.F., Korach, K.S. and Risbridger, G.P., 2002. Prostate phenotypes in estrogen-modulated transgenic mice. *Trends in Endocrinology & Metabolism*, **13**, 163–168

Pearce, S.T. and Jordan, V.C., 2004. The biological role of estrogen receptors α and β in cancer. *Critical Reviews in Oncology Hematology*, **50**, 3–22

Stocchi, R., Rea, S., Cecchini, S. and Loschi, A.R., 2004. Screening istologico e immunoistochimico della prostata con anticorpi anti-citocheratine nel controllo dell'uso di anabolizzanti nel bovino. *Atti XIV Convegno Nazionale AIVI, Vicoforte (CN)*, 325–330.

Weihua, Z., Warner, M. and Gustafsson, J.Å., 2002. Estrogen receptor beta in the prostate. *Molecular and Cellular Endocrinology*, **193**, 1–5