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Progesterone receptor up-regulation: a diagnostic tool for the illicit use of oestrogens in adult beef cattle

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The monitoring of gene regulation via mRNA levels to detect anabolic sex steroid administration in cattle is a novel approach to detecting the illicit treatment of livestock in meat production. A previous study revealed that progesterone receptor (PR) gene expression levels were increased in the bulbourethral glands and prostates of 17 β -oestradiol-treated prepubertal calves, suggesting that the PR can be used as a specific molecular biomarker for oestrogen treatment. The aim of this study was to verify the specificity and applicability of the PR to detect the illegal use of 17 β -oestradiol in sexually mature beef cattle. Accessory sex glands were sampled from 42 male beef cattle that were divided into six experimental groups, including two control groups, K1 and K2. Group A cattle were treated with 17 β -oestradiol (five weekly intramuscular doses of 20 mg), and group B cattle were treated with dexamethasone (40 daily doses of 0.7 mg per os). Group C cattle received an implant of Revalor-200 (200 mg of trenbolone acetate and 20 mg of 17 β -oestradiol), and group D cattle received Revalor-200 plus dexamethasone (0.7 mg daily per os). 17 β -Oestradiol, either alone or in combination with other steroids, up-regulated the PR gene and protein expression, even in the absence of detectable histological changes in the accessory sex glands, confirming the high sensitivity of PR gene expression as an indirect diagnostic screening tool to detect illicit oestrogen treatment in sexually mature male bovine.

Keywords: progesterone receptor; gene expression; beef cattle; 17 β -oestradiol; growth promoters

Introduction

Drugs with anabolic effects have been used to enhance feed conversion, growth rates and lean tissue depositions in stock farming for several decades (Cacciatore et al. 2009). There are multiple lines of evidence linking sex steroids, including testosterone and oestradiol in particular, to increased muscle mass in mammals. Testosterone has been demonstrated to induce muscle hypertrophy (Sinha-Hikim et al. 2003), and oestrogen has been demonstrated to stimulate muscle repair and regeneration processes (Enns and Tiidus 2010). In addition to natural steroids, synthetic derivatives, such as the glucocorticoid dexamethasone (DEX), are often administered in combination with sexual steroids and 2-agonists in young cattle to enhance re-partitioning effects of these drugs (Abraham et al. 2004). The use of synthetic steroids is banned in the EU under Directive 88/146/EEC (Stephany 2001), but in ruminants, DEX can be administered legally to treat inflammation, shock, stress and metabolic disease (Cantiello et al. 2009). The detection of the illicit use of such agents is largely based on the direct detection of drug residues via highly sensitive LC–MS/MS and GC–MS analyses of various matrices, such as muscle tissue, blood and urine (De Brabander et al. 2007). Although mass spectrometry-based residue analysis is very sensitive, it is expensive and currently unable to fully encompass the ever-expanding range of growth-promoting compounds that are often administered as cocktails of several low-dose drugs (Courtheyn et al. 2002). The advent of new designer drugs, together with the illicit use of hormones, has highlighted the necessity of the development of new techniques to improve the detection of growth promoter abuse in livestock. Bioassays have been developed as screening tools for the determination of hormonal activities in a variety of matrices, such as urine (Divari et al. 2010), feed (Bovee et al. 2006, 2009), hair (Becue et al. 2011) and blood (Mooney et al. 2009). In addition, indirect screening methods have been used to detect growth promoter use in farm animals at the histopathological level (Biolatti et al. 2003; Groot et al. 2007). These screening methods depend on being able to identify, with relatively low costs and in a short time, the morphological and functional changes in tissues caused by the anabolic drugs. The accessory sex glands, such as the bulbourethral, prostate, major vestibular and mammary glands, have been demonstrated to be very sensitive to the action of sexual hormones, even at low levels, resulting in clear histological changes (Biolatti et al. 2003; Castagnaro and Poppi 2006; Biolatti 2009).

Recently, a novel “omics” approach has been developed (Feng et al. 2008; Schneider and Orchard 2011) to clarify the correlations and dependencies between molecular components in biological systems. “Omics” sciences include a wide variety of fields including genomics, transcriptomics, proteomics and metabolomics. In particular, transcriptomics is the study of the transcriptome, which is also referred to as the expression profiling changes at specific times under specific environmental conditions in a given tissue or cell population. The screening of the regulation and function of anabolic sex steroids via modified gene expression levels in various tissues could be a new approach for detecting treatments with unknown drugs or newly combined drug cocktails (Reiter et al. 2007). The ligand-stimulated steroid hormone receptors, such as the oestrogen receptor (ER), androgen receptor (AR), glucocorticoid receptor (GR), progesterone receptor (PR), and mineralocorticoid receptor, form complexes with co-activators and general transcription factors as well as recognise and bind hormone response elements in the regulatory regions of various hormone-responsive genes. This induces the modulation of target gene transcription (Horie-Inoue et al. 2006).

A number of novel gene expression profiles have been examined in different bovine tissues, and some of these findings could be applicable in the field as routine screening methods (Toffolatti et al. 2006; Reiter et al. 2007; Carraro et al. 2009; Giantin et al. 2010; Lopparelli et al. 2010, 2011). Recently, De Maria et al. (2010) investigated PR mRNA expression in bulbourethral glands as a potential biomarker of illicit 17 α -oestradiol administration in veal calves and found that it may be a promising new approach to improve the current residue control programmes. Furthermore, PR gene over-expression following 17 α -oestradiol administration can be detected for up to 7 days after the treatment is discontinued, whereas chemical methods can detect residues for only up to a few hours after administration. Because PR gene over-expression has only been reported in prepubertal veal calves, the aim of this work was to investigate its behaviour in the prostate and bulbourethral glands of adult male cattle after treatment with 17 α -oestradiol compared to groups of animals treated with glucocorticoids or sex steroid cocktails and controls. This method was primarily developed to facilitate the detection of the abuse of natural steroids, such as oestradiol and testosterone, in adult animals, which is normally difficult due to the high physiological production of endogenous hormones.

Because cytokeratins are involved in the genesis of hyperplastic and metaplastic lesions in the prostate and bulbourethral glands of cattle treated with oestrogens, particularly 17 α -oestradiol (Lommen and Groot 1993; Groot et al. 2007; De Maria et al. 2010; Pezzolato et al. 2011), cytokeratin 5 (KRT5) gene expression of the accessory sex glands of experimental animals was also examined to investigate the correlation between PR and KRT5 gene expression and epithelial lesions in the same glands of treated animals.

Materials and methods

Experimental design

In trial 1, 19 male Charollais beef cattle ranging from 17 to 22 months in age were divided into three groups as follows: group A cattle (n = 6) were administered five weekly intramuscular doses of 20 mg of 17 α -oestradiol, group B cattle (n = 6) were administered DEX (40 doses of 0.7 mg per day per os) and group K1 cattle (n = 6) were untreated.

In trial 2, 24 Friesian male beef cattle ranging from 13 to 20 months in age were divided into three groups as follows: group C cattle (n = 8) were administered 200 mg of trenbolone acetate and 20 mg of 17 α -oestradiol (Revalor-200, Intervet, USA) as a subcutaneous implant for 89 days plus DEX per os (0.7 mg/day/animal) for 40 days, group D cattle (n = 8) received Revalor-200 for 89 days in the same manner and group K2 cattle (n = 8) were untreated.

The animals were housed in boxes that measured 10 x 15 m with concrete floors lacking litter or lateral partitions. All groups were housed in separate boxes and fed a concentrated diet consisting of corn silage, corn, hay, and a commercial protein supplement; water was supplied ad libitum. Hormone dosages were chosen according to the literature (Vanderwal et al. 1975; Meyer 2001; Cannizzo et al. 2008; De Maria et al. 2010).

In trial 1, animals were euthanised after 7-day drug withdrawals; in trial 2, euthanasia was performed 5 days after the last DEX treatment, with the implant remaining in place until euthanasia. Target tissues were collected at slaughter and preserved for histological and molecular analyses.

This experiment was authorised by the Italian Ministry of Health and the Ethics Committee of the University of Turin. Carcasses of treated animals were destroyed (2003/74/CE - DL 16 March 2006, 2 n. 158).

Tissue sampling and processing

Bulbourethral glands and prostates were collected from each animal. Tissue samples were fixed in 10% neutral buffered formalin at room temperature, processed and paraffin-embedded according to routine histological procedures. Representative sections of each sample were stained with haematoxylin–eosin (HE).

Samples from all tissues were fixed in 5–10 volumes of RNAlater Solution (Ambion, USA) for molecular studies and stored at 4 C overnight, after which the supernatants were removed and frozen at -80 C for long-term storage.

Immunohistochemistry

Immunohistochemical staining of the basal cells was performed on the prostate and bulbourethral sections after the inactivation of endogenous peroxidase and antigen retrieval with 10 mM citrate buffer, pH 6.0 in a water bath at 98 C for 40 min. The sections were subsequently incubated with the following antibodies: anti-PR monoclonal antibody (1:70) (Ab-2 clone hPRa, Thermo Scientific, USA) for 60 min at room temperature, anti-ER (1:50) (clone 1 D5, Dako, Denmark) for 90 min at room temperature and anti-KRT5 (1:15) (clone RCK 103, Euro-diagnostica, Sweden) for 90 min at room temperature. The immunostaining was visualised with the HRP universal polymer (ABC Vectastain, Vector Lab, USA) by a Dako Autostainer.

Total RNA extractions and quantitative expression analyses of PR, ER and KRT5

Several milligrams of each tissue were disrupted using a TissueLyser II (Qiagen, Germany) with stainless steel beads in 1 ml of Trizol (Invitrogen, USA) and a DNA-free kit (Ambion, USA). RNA concentrations were determined spectrophotometrically, and RNA integrities were evaluated using an automated electrophoresis station (Experion Instrument, BioRad, USA). Total RNA was purified from residual genomic DNA, and cDNA was synthesised from 1 mg of total RNA using QuantiTect Reverse Transcription (Qiagen, Germany). To determine the relative amounts of specific ER and KRT5 transcripts, the cDNA was subjected to quantitative polymerase chain reaction (qPCR) (Kubista et al. 2006) using the IQ 5 detection system (BioRad, USA) and respective gene primers in an IQ SYBR Green Supermix (BioRad, USA). Primer sequences were designed using Primer Express (vers. 1.5) and Primer 3 (vers. 0.4.0) (31) (Table 1). The cyclophilin A (PPIA) gene was used as a housekeeping gene control, as previously reported (De Maria et al. 2010). To determine the amount of PR transcripts, the cDNA was subjected to qPCR using TaqMan probes that were specific for the PR and PPIA genes and labelled at the 5' ends with fluorescein and hexachloro-fluorescein, respectively (Table 1). The probes and relative primers were designed by the software geNorm, which was available online (<http://medgen.ugent.be/genorm>).

The relative levels of gene expression were calculated using a relative quantification assay according to the comparative Ct method (DDCt method) when the primer efficiencies were similar; before each qPCR, we performed a validation experiment to demonstrate that the amplification efficiencies of the target and reference genes were approximately equal. Then, the relative abundances of each transcript, which were normalised to the endogenous housekeeping gene transcript (PPIA) and relative to the control sample, was recorded as 2^{-DDCt} (fold increase), where $DDCt = \frac{1}{2} (DCt_{\text{treated sample}} - DCt_{\text{control sample}})$, and DCt is the Ct of the target gene subtracted from the Ct of the housekeeping gene (Livak and Schmittgen 2001; Pfaffl 2004; Wong and Medrano 2005).

Statistical analyses

Statistical tests were performed using GraphPad InStat (vers. 3.05) statistical software (GraphPad Inc., San Diego, CA, USA). The gene expression of PR, ER and KRT5 was analysed by one-way analysis of variance followed by the Bonferroni post-hoc test; when Bartlett's test suggested that the differences between the standard deviations of each group were significant, the non-parametric Kruskal–Wallis test and Dunn's post hoc test were applied.

Grubbs' test was used to reveal potential outliers. A p value of ≤ 0.05 was considered statistically significant.

Gene	Primer	Sequence	GenBank no.
ER	Forward	5 ⁰ -ACCAACCAGTGCACGATTGA-3 ⁰	NM_001001443.1
	Reverse	5 ^U -TTCCGTATTCCGCCTTTCAT-3 ^U	
AR	Forward	5 ^U -CTTCTCTCCACCCGATGTGTG-3 ^U	AY862875.1
	Reverse	5 ^U -TCTCCATCCAGGGACCCAT-3 ^U	
GR	Forward	5 ^U -CCATTTCTGTTACACGGTGTG-3 ^U	AY238475
	Reverse	5 ^U -CTGAACCGACAGGAATTGGT-3 ^U	
KRT5	Forward	5 ^U -GAGCCTTTGTTAGAGCAGTACATCAA-3 ^U	NM_001008663.1
	Reverse	5 ^U -CCTGCATATTCCTGAGCTCTGA-3 ^U	
PR	Forward	5 ^U -CCAGAGCCCACAGTACAGCTT-3 ^U	AY656812
	Reverse	5 ^U -CAGCTTCCACAGGTGAGGACA-3 ^U	
PPIA	TaqMan Probe	5 ^U -CAGCTGATGCTTCATCCCCACAG-3 ^U	NM_178320
	Forward	5 ^U -GCCCAACACAAATGGTT-3 ^U	
	Reverse	5 ^U -CCCTTTTCACCTTGCCAAAAG-3 ^U	
	TaqMan Probe	5 ⁰ -TGCTTGCCATCCAACCACTCAGTC-3 ⁰	

Results

Gross findings and histopathology

Macroscopic examination of the accessory sex glands revealed no differences among the experimental and control groups. In contrast, histological evaluation of group A cattle revealed marked hyperplasia and metaplasia in the prostatic urothelium and similar but less severe lesions in the glandular epithelium that were mostly localised to the peripheral portion of the gland, whereas the epithelium of the ducts had similar features as those of the urothelium. In the bulbourethral glands, diffuse hypersecretion, cysts, hyperplasia and focal metaplasia were detected.

No hyperplasia or metaplasia was detected in the other experimental groups (B, C, D) or controls (K1, K2) (Figure 1), excluding one animal from group D that had focal metaplasia in its urothelium. Lymphocytic infiltration in the urethral epithelium and glandular portion that was independent of the hormonal treatment was observed in a few animals from all groups.

Immunohistochemistry

Immunohistochemical staining revealed widespread nuclear PR expression in the bulbourethral gland, prostate epithelium and urothelium in all groups; however, this expression was markedly increased in the animals of groups A, C and D. In group A, PR was strongly expressed in the epithelial hyperplastic and metaplastic areas of the prostate and bulbourethral glands, and the positive areas were mostly localised to the basal layer of the urothelium and glandular duct epithelium. Animals in groups C and D exhibited diffuse PR-positive areas in both of the glands, the epithelium and basal layer of the urothelium, whereas PR-positive areas were mostly apical in the glandular ducts (Figure 2).

In contrast, PR protein was generally weak and observed only in the periphery of the glands in the control and DEX-treated animals. The nuclei of the urothelium were also diffuse and weakly positive, whereas the epithelial ducts exhibited weak apical PR expression (Figure 3).

Furthermore, in the cattle in groups A, C and D, PR protein expression was increased in the smooth muscle cells, especially those around the urethra and ducts of the prostate.

ER staining was evident in all nuclei of the excretory ducts and glandular epithelia of the prostate in all animals, but ER was more highly expressed in the urothelium than in other areas of the gland. ER staining of the bulbourethral glands was weaker than that of the prostate and was observed in all animals (data not shown).

In both the prostate and bulbourethral glands of group A animals, KRT5 staining revealed the over-expression of the cytoplasmic KRT5 protein in the hyperplastic and metaplastic epithelia. In the urethra in particular, the entire urothelium was diffusely and strongly stained (Figure 4).

In the prostates of animals in groups B, C and D, KRT5 positivity was limited to scattered basal cells in both the glandular epithelium and urothelium, which was similar to the findings in the control glands (Figure 4). The same features were observed in the bulbourethral gland, in which the basal epithelium of the excretory ducts

and basal cells of the glandular acini exhibited staining patterns that were similar to the control staining patterns (Figure 4).

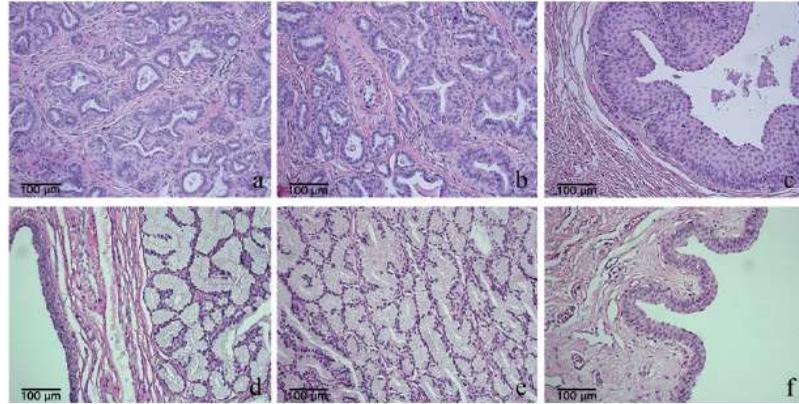


Figure 1. Trial 1: Accessory sex glands of 17β -oestradiol-treated beef cattle (group A) and control cattle (K1). The bulbourethral and prostate glands of group A animals exhibited epithelial hyperplasia and metaplasia in the secreting portions (a and b) and metaplasia of the urothelium (c). The bulbourethral (d) and prostate glands (e) and urethra (f) of group K1 animals did not have lesions. HE (200 \times).

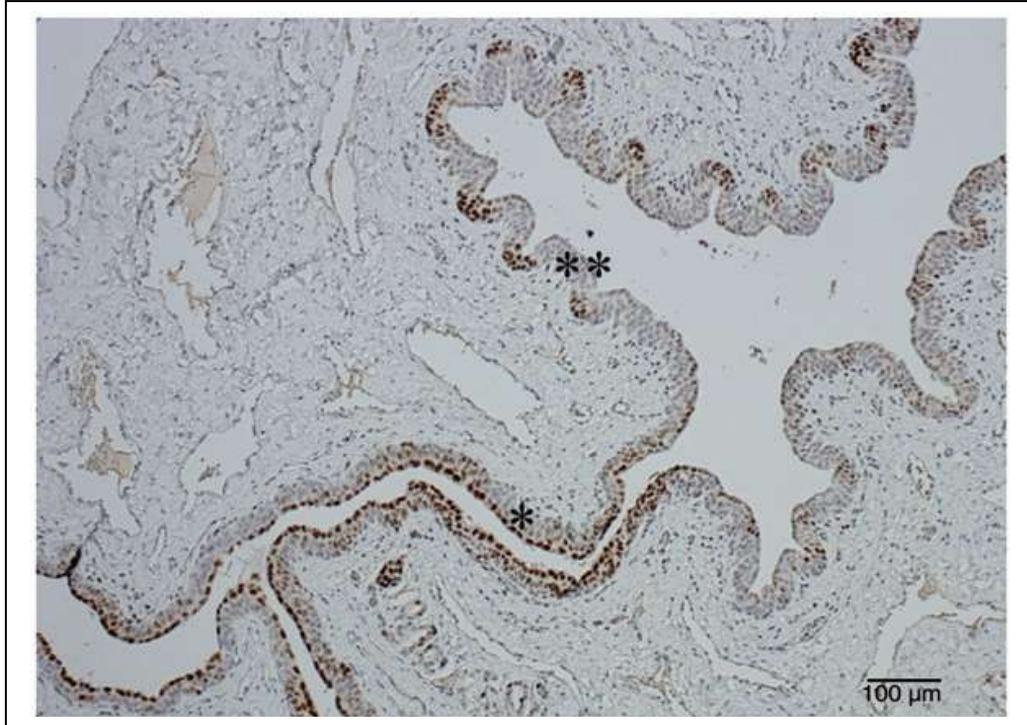


Figure 2. Trial 2, group C: The epithelium of the prostate glandular duct (*) inserting into the urethra (**) exhibited strong superficial PR positivity, whereas the PR protein expression in the urethra was higher in the basal layers of the urothelium. Immunohistochemistry (100_).

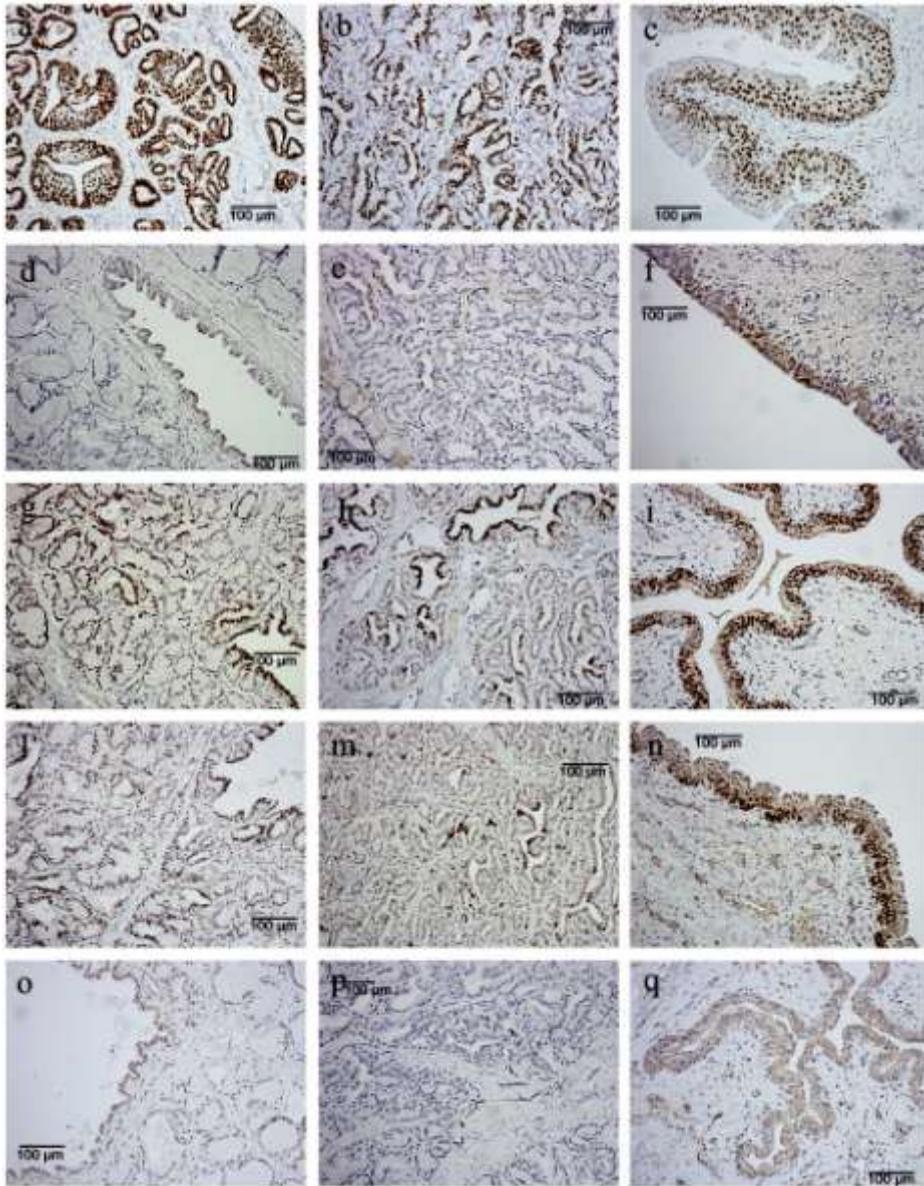


Figure 3. Trial 1 and Trial 2: Immunohistochemistry staining patterns of PR in the accessory sex glands of experimental animals. The bulbourethral (a) and prostate glandular epithelia (b) of group A cattle exhibited strong PR nuclear positivity, and PR positivity was observed, although less strongly, in the same regions of the groups C (g and l) and D glands (h and m). The urothelia of groups A (c), C (i) and D (n) cattle exhibited strong PR positivity, particularly in the basal layers. The bulbourethral and prostate glands and urothelium of group B (d, e and f) and control cattle (o, p and q) exhibited weak staining for the PR protein. Immunohistochemistry (200).

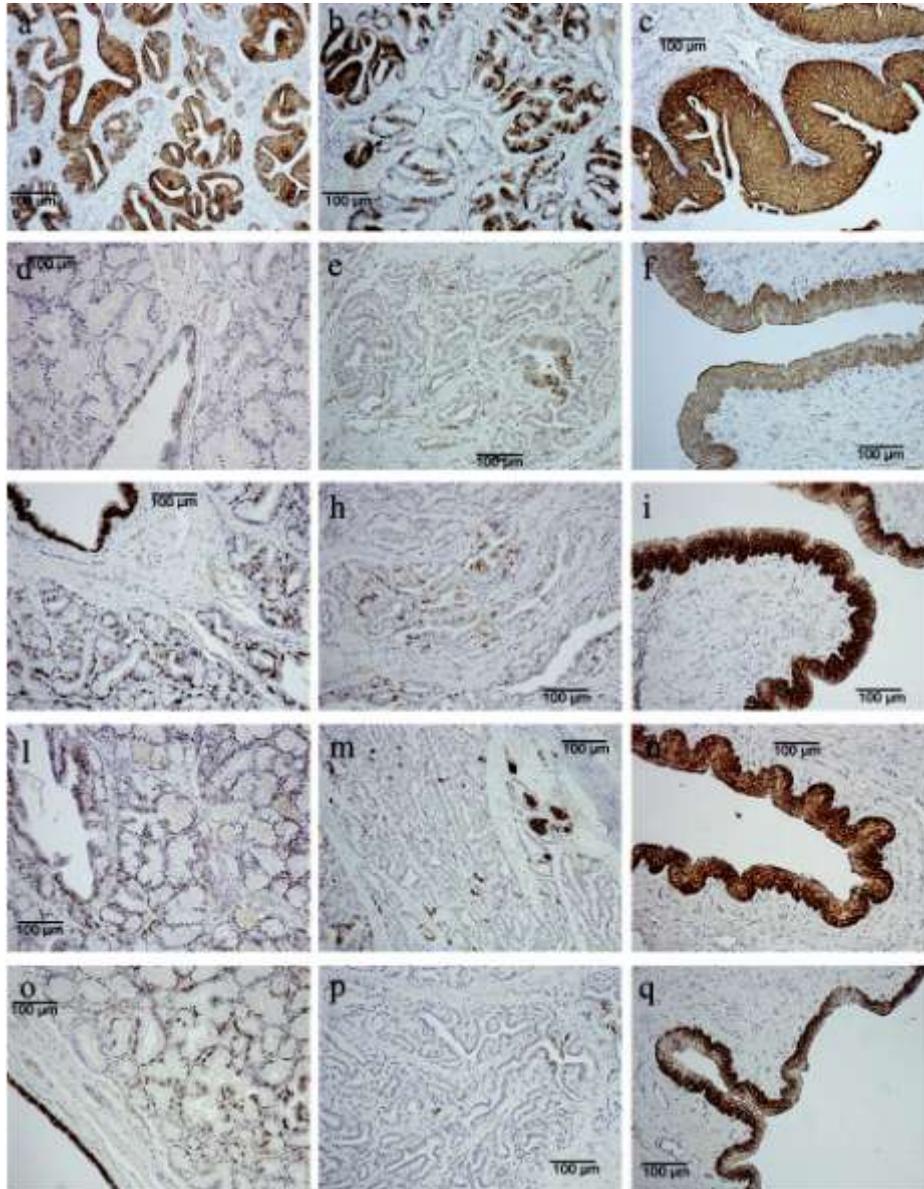


Figure 4. Trial 1 and Trial 2: Immunohistochemistry of KRT5 in the accessory sex glands of the experimental animals. The cytoplasm of the bulbourethral (a) and prostate (b) gland epithelial cells urothelium (c) of group A cattle were diffusely and strongly positive for KRT5. In all of the other groups, PR positivity was limited to scattered basal cells of the bulbourethral and prostate glands (d and e: group B; g and h: group C; l and m: group D; o and p: group K1). KRT5 protein expression in the urothelia of cattle in groups B (f), C (i), D (n), K1 (q) and K2 was principally localised to the basal layer. Immunohistochemistry (200).

Molecular analysis

qPCR specific for PR, ER, AR, GR, and KRT5 was performed to examine the expression levels of the corresponding genes in the bulbourethral and prostate glands. Tables 2 and 3 reveal significant up-regulation in the levels of PR, ER and KRT5 in both glands of group A animals that exhibited histological changes.

PR gene expression was also elevated in the bulbourethral glands of animals in groups C and D, but the gene expression of ER and KRT5 did not change; the same expression profile was observed in the prostate gland, in which PR gene up-regulation was less marked. In particular, the bulbourethral and prostate glands of group C animals had higher PR expression than group D animals. The tested genes were not regulated by DEX in group B animals, and the expression of AR and GR in particular did not change in any experimental group compared to those in the controls.

Table 2. Fold gene expression changes in the five genes considered in the bulbourethral glands of the animal from the four experimental groups versus each control (group K1 for A and B group and K2 for C and D groups, to whom a control value of 1 was assigned).

Genes	Normalised fold expression ($2^{-\text{DDCt}}$)			
	A	B	C	D
PR	23.4221.49***	0.831.40	34.6960.82***	20.1930.08***
ER	3.092.04***	0.80.48	1.931.79	2.392.44
AR	2.011.33	0.990.73	2.592.86	1.331.15
GR	2.171.59	0.810.93	1.731.69	1.301.17
KRT5	9.9413.05**	0.840.48	1.811.87	1.131.36

Note: **p \leq 0.01; ***p \leq 0.001.

Table 3. Fold gene expression changes in the five genes considered in the prostate gland of the animal from the four experimental groups versus each control (group K1 for A and B group and K2 for C and D groups, to whom a control value of 1 was assigned).

Genes	Normalised fold expression ($2^{-\text{DDCt}}$)			
	A	B	C	D
PR	25.0715.90***	0.861.07	7.1111.35*	5.388.43*
ER	6.376.96**	1.571.58	1.071.28	1.461.96
AR	1.700.94	1.401.51	0.781.06	1.151.39
GR	1.821.25	1.041.58	0.240.52	0.851.12
KRT5	5.186.74*	0.740.92	1.211.25	2.103.33

Note: *p \leq 0.05; **p \leq 0.01; ***p \leq 0.001.

Discussion

As described in the Introduction, the alternative indirect screening methods that are used to detect hormonal treatment in farm animals are useful for direct analyses of residues. The present study examined a novel strategy based on PR gene expression in adult male accessory sex glands in response to 17 -oestradiol that was administered either alone or in combination with other molecules to reveal growth promoter abuse. In this experiment, in addition to the typical epithelial hyperplastic and metaplastic changes that were observed in the prostate, urethra and bulbourethral glands (Biolatti et al. 2003; Groot et al. 2007; De Maria et al. 2010), 17 -oestradiol induced significant PR transcriptional up-regulation in the prostate (25.07-fold) and bulbourethral glands (23.42-fold). These data were confirmed by a protein expression analysis using immunohistochemistry. Moreover, 17 -oestradiol specifically induced the transcriptional up-regulation of KRT5 and ER . KRT5 is involved in the genesis of epithelial hyperplastic and metaplastic lesions in accessory sex glands. No expression changes were observed in the GR and AR. This is explained by the fact that 17 -oestradiol only binds to ER , which is one of two specific ERs (and) that are members of the nuclear receptor super-family of ligand-regulated transcription factors (Pettersson and Gustafsson 2001). The complex formed by ER and an oestrogen co-migrates from the cytosol into the nucleus, interacting with specific DNA sequences that directly or indirectly activate or repress the target genes (Björnström and Sjöberg 2005; Glidewell-Kennedy et al. 2005; Prins et al. 2006). The PR and ER genes are regulated by 17 -oestradiol (Ing and Tornesi 1997; Hess et al. 2001; Luconi et al. 2002). In breast cancer and other oestrogen-regulated neoplasias, such as prostate cancer, the PR is a widely accepted marker that indicates oestrogen action (Bonkhoff et al. 2001). In the mouse model, exposure to oestrogens induces metaplasia of the urethral epithelium, and PR over-expression (Risbridger et al. 2001) is clearly detectable by immunohistochemistry. Therefore, PR gene expression is oestrogen-dependent; in fact, group B animals treated with DEX did not exhibit any alterations in the mRNA levels of the PR gene in their accessory sex glands. Furthermore, in support of the previous finding, testosterone administration in veal calves did not up-regulate PR and ER (De Maria et al. 2010). It is very likely that the innovative results of this work will be applied to the detection of PR over-expression in adult male bovine, such as beef cattle, which have been treated with 17 -oestradiol, either alone or in combination with other hormones, such as androgens and glucocorticoids.

In this case, despite the low 17 -oestradiol concentrations, PR mRNA and protein levels in the accessory sex glands were significantly higher in 17 -oestradiol-treated animals than in the untreated animals. In contrast, the bulbourethral and prostate glands of the animals that were treated with the drug cocktail did not exhibit any histological lesions. Consequently, KRT5 gene and protein expression remained similar to those of the control. Similar results were observed for ER . It is likely that the presence of molecules other than 17 -oestradiol and its low concentration in the implants modulated the effects of the oestrogen hormone.

Immunohistochemistry with specific antibodies against the PR, ER , and KRT5 proteins performed on bulbourethral and prostate glands permitted the verification of the increased expression and tissue localisations of the target proteins. The increased areas of PR positivity in the accessory sex glands of animals in groups A, C, and D confirmed the gene expression data. The

proteins were expressed mainly in the urothelial basal cell layers as well as hyperplastic and metaplastic cells of the glandular portion. This was more evident in group A cattle, in which histological changes were observed. In the animals treated with the drug cocktail, the urethral epithelium appeared more sensitive to the action of oestrogen and exhibited strongly positive regions, particularly in the basal layers, whereas in the glandular ducts, PR over-expression was only observed in the epithelial cell nuclei of the superficial layers and scattered groups of cells. In the bulbourethral glands, PR protein staining was less evident, which was probably because the nucleus is flattened at the base of mature secreting cells. In all of the groups of animals that were treated either with 17 β -oestradiol alone or in cocktails, the smooth muscle cells surrounding the urethra and ducts of the prostate were positive for PR staining, confirming the physiological regulatory effects of progesterone on smooth cell contraction that are analogous to its progestinic effects on the oviductal and uterine smooth muscle cells (Schleicher et al. 1993). Unlike PR protein expression, KRT5 expression increased only in hyper-plastic and metaplastic cells, whereas in cattle in groups C and D, in which histological changes were absent, KRT5 protein levels did not increase, which was confirmed at the mRNA level. This observation leads to the conclusion that the PR is an effective biomarker for previous oestrogen hormone treatment, even at low dosages and in the absence of clear target tissue alterations.

17 β -Oestradiol induced a moderate up-regulation of ER that was not sufficient to be detected by immunohistochemical staining (data not shown), and the administration of other molecules in combination with 17 β -oestradiol inhibited the expected over-expression of its receptor in the accessory sex glands.

Trenbolone and DEX did not change the gene expression of AR and GR in the prostate and bulbourethral glands, but the combination of DEX and Revalor-200 slightly increased PR expression. An explanation for these increased mRNA levels could be related to the promoter structure of the PR gene. Petz and Nardulli (2002) demonstrated that an oestrogen response element half-site and two adjacent Sp1 sites in the human PR gene are involved in 17 β -oestradiol-mediated activation of the PR gene. As GRs also interact with Sp1-binding sites indirectly via the transcription factor Sp1 to activate the transcription of the gene target (Ou et al. 2006), DEX could enhance the up-regulation of PR gene expression in combination with 17 β -oestradiol.

These data support the use of PR gene expression in accessory sex glands in a screening analysis to identify animals that have been treated with drug cocktails. Moreover, this over-expression may not necessarily be linked to histological lesions and, consequently, the PR mRNA levels could also be utilised to support the histological test that has already been adopted for screening by the Italian Ministry of Health.

Conclusions

These results demonstrate that 17 β -oestradiol specifically induces an increase in the PR mRNA levels in the prostate and bulbourethral glands of sexually mature adult beef cattle. PR over-expression can be induced by 17 β -oestradiol alone or in combination with other agents and is independent of histological changes. This finding permits the application of this indirect biomarker in a screening test to detect the illicit treatment of male cattle with oestrogens in the official residue-

monitoring programmes.

The detection of oestradiol abuse by PR up-regulation is, therefore, a promising tool to further ensure food safety and quality, considering that changes in the expression of this gene may persist for up to a week after the suspension of 17 -oestradiol treatment.

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