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### ► To cite this version:

Maria Groot, Johan Lasaroms, Eric van Bennekom, Thijs Meijer, Ester Vinyeta, et al.. Detection of illegal treatment of barrows with nandrolone ester; effects on growth, histology and residue levels in urine and hair. Food Additives and Contaminants, 2012, pp.1. 10.1080/19440049.2011.647097 . hal-00783341

**HAL Id: hal-00783341**

**<https://hal.science/hal-00783341>**

Submitted on 1 Feb 2013

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**Detection of illegal treatment of barrows with nandrolone ester; effects on growth, histology and residue levels in urine and hair**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-451.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	30-Nov-2011
Complete List of Authors:	Groot, Maria ; RIKILT Institute of Food Safety,The Netherlands, Bioanalysis and Toxicology Lasaroms, Johan; RIKILT - Institute of Food Safety, Analysis and Development Van Bennekom, Eric; RIKILT - Institute of Food Safety, Analysis and Development Meijer, Thijs; RIKILT - Institute of Food Safety, Analysis and Development Vinyeta, Ester; Schothorst Feed Research B.V., Van der Klis, Jan-Dirk; Schothorst Feed Research B.V., Nielen, Michel; Wageningen University, 3Laboratory of Organic Chemistry; RIKILT - Institute of Food Safety, Analysis and Development
Methods/Techniques:	Chromatography - LC/MS, Bioassay, Chromatography - GC/MS
Additives/Contaminants:	Drug residues - hormones
Food Types:	Animal products – meat
Abstract:	<p>Abstract</p> <p>To evaluate the effects of 17<math>\beta</math>-19-nortestosterone (17<math>\beta</math>NT ) treatment of barrows on residue levels and on growth results, an animal experiment was conducted. Five barrows were treated 3 times during the fattening period with 17<math>\beta</math>NT phenylpropionate (Nandrosol, nandrolone phenylpropionate 50 mg/ml,1 mg/kg BW). Another 5 barrows were not treated, and 5 boars (not treated) were kept as a positive control. Boars and treated barrows showed 13 % and 9 % better growth than not-treated barrows, leading to mean final body weights of 121.6, 117.8 and 109.0 kg respectively. Moreover the bulbourethral glands of the treated barrows were 3 times heavier than the not-treated barrows. The histology of the prostate and bulbourethral gland of the treated barrows was comparable to the boars, whereas the control barrows showed atrophic glands. Levels of 17<math>\beta</math>NT ester in hair from treated barrows were high, whereas</p>

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	boars and non-treated barrows did not show levels above LLQ. It is concluded that analysis of hair can provide a method for detection of illegal treatment with 17 $\beta$ NT ester in barrows. The size of the bulbourethral gland can be used for screening in the slaughterhouse.

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3 **1 Detection of illegal treatment of barrows with nandrolone ester;**  
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5 **2 effects on growth, histology and residue levels in urine and hair**  
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11  
12 van der Klis<sup>2</sup> and M.W.F. Nielen<sup>1,3</sup>  
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32  
33 *Abstract*

34  
35 To evaluate the effects of 17 $\beta$ -19-nortestosterone (17 $\beta$ NT) treatment of barrows on  
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37 residue levels and on growth results, an animal experiment was conducted. Five  
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39 barrows were treated 3 times during the fattening period with 17 $\beta$ NT  
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41 phenylpropionate (Nandrosol, nandrolone phenylpropionate 50 mg/ml, 1 mg/kg BW).  
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1 were high, whereas boars and non-treated barrows did not show levels above LLQ. It  
2 is concluded that analysis of hair can provide a method for detection of illegal  
3 treatment with  $17\beta$ NT ester in barrows. The size of the bulbourethral gland can be  
4 used for screening in the slaughterhouse.

5  
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8  
9 *Key words: 17 $\beta$ -19-nortestosterone, barrows, hair analysis, histology, urine analysis*

## 10 11 **Introduction**

12 In male pigs  $17\beta$ -19-nortestosterone ( $17\beta$ NT) is a naturally occurring compound in  
13 urine (Poelmans *et al.*, 2005). In boars high levels can be measured but in barrows  
14 and gilts only low levels occur. In practice however sometimes high levels of  $17\beta$ NT  
15 are found in urine from barrows. This can be due to cryptorchid animals, having one  
16 testicle in the abdomen that produces hormones. But illegal treatment with  $17\beta$ NT for  
17 growth promotion is also a possibility, since 19-nortestosterone (nandrolone) has a  
18 long history for abuse as growth promoter in livestock (Rico, A.G., 1983).

19 The use of hormonal substances for fattening purposes in livestock is banned in the  
20 EU since 1988 according to Council Directive 96/22 EC (Offic. J. Eu. Comm., 1996).  
21 Steroids having estrogenic, androgenic, progestagenic action are forbidden as well as  
22 thyreostatics and  $\beta$ -agonists. Residue analysis on target compounds is carried out by  
23 control laboratories to enforce the ban.

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3 1 Some hormones that were considered as exogenic appeared to occur naturally in some  
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5 2 species (Scarth *et al.*, 2009). This applies to  $17\beta$ NT which occurs in urine of stallions  
6  
7 3 (Houghton *et al.*, 1994) and in urine from boars (Debruyckere *et al.*, 1990).  
8  
9 4 Boars grow faster and show a better feed conversion with 20 to 30% less fat than  
10  
11 5 barrows (Field, 1971; Mulvaney *et al.*, 1988; Kudson *et al.*, 1985). Gonadally intact  
12  
13 6 males also have 8 to 15% greater muscle mass than castrated males (Knudson *et al.*,  
14  
15 7 1885; Prescott & Lamming, 1967). In boars, testis and accessory genital glands are  
16  
17 8 normally developed, whereas barrows are castrated at a young age and have atrophied  
18  
19 9 accessory genital glands. Treatment with androgens leads to increase in growth of the  
20  
21 10 seminal vesicles and bulbourethral glands in barrows (Lauwers *et al.*, 1983; Lauwers,  
22  
23 11 1984).  
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30 13 In recent years, apart from urine analysis, hair has been analyzed for the presence of  
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32 14 esters of anabolic steroids (Sauer *et al.*, 1998; Duffy *et al.*, 2009; Nielen *et al.*, 2006,  
33  
34 15 Stolker *et al.*, 2009). For growth promoting purposes steroid hormones are usually  
35  
36 16 administered with an ester side-chain attached in order to facilitate the steroid  
37  
38 17 reaching the site of activity within the animal's body. Because steroid hormones do  
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40 18 not occur naturally in an ester form, detection of intact steroid esters demonstrates  
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42 19 illegal administration of these drugs.  
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47 21 To investigate if treatment with  $17\beta$ NT is anabolic in barrows can lead to high levels  
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49 22 of  $17\beta$ NT in urine and hair we treated barrows with  $17\beta$ NT and compared them with  
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51 23 untreated barrows and untreated boars.  
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## 55 56 25 **Material and methods**

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3 1 *Experimental*  
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5 2 The animal experiment was performed by Schothorst Feed Research BV in Lelystad,  
6  
7 3 Netherlands. The trial comprised three treatments according to sex and Nandrosol  
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9 4 injection: 1) non-injected boars, 2) non-injected barrows and 3) barrows injected with  
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11 5 17 $\beta$ NT phenylpropionate (nandrolone phenylpropionate 50 mg/ml, 1 mg/kg BW,  
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13 6 Nandrosol, REG NL 10067, Alfasan, The Netherlands). A total of 15 pigs were  
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15 7 distributed in 5 replicates per treatment group.  
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20  
21 9 Experimental animals were grower pigs (Tempo x (Great Yorkshire x Finnish  
22  
23 10 Landrace)) of 60-70 days of age at the start of the experiment and approximately 25  
24  
25 11 kg live body weight. Pigs were housed in three separate pens (one pen/treatment) of  
26  
27 12 1.80 x 1.00 m. One spare pig per treatment was also allocated within the experimental  
28  
29 13 pigs and the control barrows and was fed and weighed in case a pen mate dropped out.  
30  
31 14 The animals were fed ad libitum and had free access to water. The animals were fed  
32  
33 15 commercial growing/finishing diets (Arkevaart-Twente Coöperatie, Nijkerk, The  
34  
35 16 Netherlands) commonly used in Schothorst Feed Research in practical conditions.  
36  
37 17 The animals were injected at 19, 47 and 75 days after the start of the experiment and  
38  
39 18 were slaughtered on day 93 of the experiment. Once a month urine and hair was  
40  
41 19 sampled and every two weeks the animals were weighed. Sampling times for hair and  
42  
43 20 urine were day 12, 33, 61 and 81, respectively time points 1, 2, 3 and 4. All pigs were  
44  
45 21 delivered to a commercial slaughterhouse at a body weight around 100-115 kg.  
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47  
48 22 Treated animals were condemned for destruction. This study was approved by the  
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50 23 Ethical Committee of Animal Science Group Lelystad, part of Wageningen  
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52 24 University Research Centre, Netherlands.  
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3 1 *Histology*  
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5 2 At slaughter the animals were inspected for gross pathology. Testes (only in boars),  
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7 3 prostate and bulbourethral gland were sampled for histological investigation. After  
8  
9 4 weighing the bulbourethral glands, samples of the prostate and bulbourethral gland  
10  
11 5 were immediately fixed in 10 % phosphate buffered formalin, whereas samples of the  
12  
13 6 testis were fixed in Bouin Hollandais fixative, which gives a better morphology. After  
14  
15 7 the usual dehydration series with alcohols and xylol, the tissues were embedded in  
16  
17 8 paraffin. Sections, 5 µm thick, were made using a rotating microtome. Sections were  
18  
19 9 stained with heamatoxylin-eosin (HE) according to Mayer (Bancroft & Stevens, 1990)  
20  
21 10 for routine staining and the prostate and bulbourethral gland were also stained for  
22  
23 11 mucins with the combined Alcian Blue/periodic Acid Schiff (ABPAS) technique  
24  
25 12 staining both acidic (blue) and neutral mucins (red) (Bancroft & Stevens, 1990).  
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32 14 *Chemicals*  
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34 15 The chemicals and solutions used were analytical reagent grade. Water was purified  
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36 16 using a Milli-Q system from Millipore (Bedford, MA, USA). 17βNT  
37  
38 17 phenylpropionate ester and 17βNT were obtained from Steraloids (Newport, Rhode  
39  
40 18 Island, U.S.A.). The isotope-labeled internal standard d<sub>3</sub>-testosterone  
41  
42 19 phenylpropionate ester and d<sub>3</sub>-17βNT were custom-synthesized at Wageningen  
43  
44 20 University and Research Centre (Wageningen, The Netherlands). Stock solutions  
45  
46 21 were prepared in methanol at 1 mg/ml. Bondelut LRC-C18 solid-phase extraction  
47  
48 22 (SPE) columns (100 mg and 500 mg) were from Varian (Harbor City, CA, USA) and  
49  
50 23 ) and IST Isolute NH<sub>2</sub> solid-phase extraction (SPE) columns (100 mg) were from  
51  
52 24 Sopachem (Eke, Belgium). β-Glucuronidase/arylsulfatase (from Helix Pomatia) was  
53  
54 25 obtained from Merck (Darmstadt, Germany).  
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3 1 *Sample preparation*  
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5 2 The hair samples were washed with water and dried for 24 hours in an oven at 60°C.  
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7 3 Then the hair samples were cut in 0.5 cm pieces using a pair of scissors and 500 mg  
8  
9 4 was pulverized in a Sartorius (Goettingen, Germany) model Mikro-dismembrator S  
10  
11 5 ball mill. From each sample two test portions of two hundred milligrams of the  
12  
13 6 pulverized hair were weighed into a plastic tube. Both test portions were spiked with  
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15 7 the internal standard d<sub>3</sub>-testosterone phenylpropionate at 10 ng/g. In addition one of  
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17 8 the test portions was spiked with 17βNT phenylpropionate at 5 ng/g. Blanks and  
18  
19 9 controls were spiked with the internal standard d<sub>3</sub>-testosterone phenylpropionate at  
20  
21 10 10 ng/g. Control hair samples were prepared by spiking blank hair with 17βNT  
22  
23 11 phenylpropionate at 0, 2.5, 5.0, 10 and 25 ng/g. Digestion of the hair was performed  
24  
25 12 by addition of 2 ml of 25 mM tris(2-carboxyethyl)phosphine (TCEP) and incubation  
26  
27 13 at room temperature for 1 hour. The tube was shaken by using a 'head-over-head'  
28  
29 14 shaker. After addition of 4 ml of methanol, the tube was centrifuged for 5 min at  
30  
31 15 1700g. Next, 4 ml of water was added and the mixture was applied to a C18 solid-  
32  
33 16 phase extraction (SPE) cartridge, pre-activated with acetonitrile, methanol and water.  
34  
35 17 The SPE-cartridge was washed with 2 ml of methanol/water (60/40 v/v) and eluted  
36  
37 18 with 2 ml of acetonitrile followed by 2 ml of ethyl acetate. The combined eluate was  
38  
39 19 evaporated to dryness under a gentle stream of nitrogen gas at 40°C and re-dissolved  
40  
41 20 in 200 μl of water-acetonitrile-methanol-formic acid (300/350/350/20, v/v/v/v). 40 μL  
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43 21 was injected into LC-MS/MS system 1.  
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51  
52 23 From each urine sample two test portions (2 ml) were transferred into a glass tube.  
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54 24 Both test portions were spiked with the internal standard d<sub>3</sub>-17β-19-nortestosterone at  
55  
56 25 2 ng/ml. In addition one of these portions was spiked with 17β-19-nortestosterone at 2  
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1 ng/ml. Blanks and controls were spiked with the internal standard  $d_3$ -17 $\beta$ -19-nortestosterone at 2 ng/ml. The control urine samples were prepared by spiking blank urine with 17 $\beta$ -19-nortestosterone at 0, 0.5, 1.0, 2.0, 3.0 and 5.0 ng/ml. Prior to hydrolysis, the urine samples were adjusted to pH 4.8. Following the addition of 10  $\mu$ L  $\beta$ -glucuronidase/arylsulfatase (ready to use, Merck), enzymatic deconjugation was carried out in a water bath at 37 °C for 16 hr. The hydrolyzed sample was applied to a C18 solid-phase extraction (SPE) cartridge, pre-activated with methanol and sodium acetate buffer. The vacuum dried SPE column was eluted with 4 ml of methanol. The eluate was applied to a NH<sub>2</sub> SPE cartridge and the eluate was evaporated to dryness under a gentle stream of nitrogen. The residue obtained was re-dissolved in 225  $\mu$ L (methanol/water 20/80 v/v) and 200  $\mu$ L was injected into a HPLC system consisting of a vacuum degasser, an autosampler, a binary pump, an UV detector and a fraction collector from Agilent (Santa Clara, California). The fraction where 17 $\beta$ -19-nortestosterone eluted was collected and evaporated to dryness under a gentle stream of nitrogen and re-dissolved in 300  $\mu$ l methanol/formic acid (0.5%) (50/250 v/v). 40  $\mu$ l was injected into LC-MS/MS system 2.

#### 18 *LC-MS/MS systems*

19 System 1: The analysis of the hair samples was carried out using a UFLC liquid chromatography system, consisting of a vacuum degasser, an auto sampler and a binary pump from Shimadzu Scientific Instruments (Colombia, MD, USA) equipped with a reversed phase Waters Acquity UPLC BEH C18 analytical column of 100\*2.1 mm and 1.7  $\mu$ m particle size kept in a column oven at 50 °C. The LC gradient (solvent A, water-acetonitrile-methanol-formic acid (300/350/350/20; v/v/v/v); solvent B, acetonitrile-methanol-formic acid (500/500/20; v/v/v)) with a flow of 0.4

1 ml/min was: 0 min 10% B; 0.1-2 min linear increase to 70% B; 2-8 min linear  
2 increase to 100%B; 8-9.5 min 100% B. Injection volume was 40  $\mu$ L. The UFLC  
3 system was connected to a AB Sciex (Concord, Ontario, Canada) mass spectrometer  
4 model QTRAP 5500 equipped with an electrospray interface operating in the positive  
5 ionization mode. IonSpray voltage was set at 5500V and the source temperature was  
6 set at 250°C. Curtain gas, gas 1 and gas 2 were 20, 55 and 60 psi respectively. The  
7 desolvation gas and the collision-induced dissociation (CID) gas was nitrogen.  
8 Entrance potential (EP) for all analytes was 10V. For 17 $\beta$ -19-nortestosterone  
9 phenylpropionate ester the most abundant MRM transition (used for the  
10 quantification) was acquired using the following conditions: precursor ion mass (m/z)  
11 407.1, Product ion mass (m/z) 105.2 with a Collision Energy of 33 (eV). A second  
12 MRM transition, used for the confirmation of the identity of 17 $\beta$ -19-nortestosterone  
13 phenylpropionate ester, was acquired using the following conditions: precursor ion  
14 mass (m/z) 407.1, Product ion mass (m/z) 133.1 with a Collision energy of 25 (eV).  
15 For d<sub>3</sub>-17 $\beta$ -testosterone phenylpropionate the most abundant MRM transition was  
16 acquired using the following conditions: precursor ion mass (m/z) 424.2, product ion  
17 mass (m/z) 105.1 with a collision energy of 30 (eV).

18  
19 The concentrations of 17 $\beta$ -19-nortestosteronephenylpropionate in hair samples were  
20 calculated using the isotope dilution method and a standard addition of 5 ng/g 17 $\beta$ -19-  
21 nortestosterone phenylpropionate to each individual sample.

22  
23 System 2: The LC-MS/MS system consisted of a Waters (Manchester, UK) Acquity  
24 UPLC model LC system equipped with a Waters model Quattro Premier XE triple-  
25 quadrupole mass spectrometer. The mass spectrometer was operated in the positive

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3 1 electrospray ionization (ESI) mode at a capillary voltage of 2.7 kV, a desolvation  
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5 2 temperature of 450 °C, a source temperature of 120 °C and a cone voltage of 30V.  
6  
7 3 The desolvation gas was nitrogen and the collision-induced dissociation (CID) gas  
8  
9 4 was argon. A Waters Acquity UPLC BEH C18 analytical column of 50\*2.1 mm and  
10  
11 5 1.7 µm particle size was used and kept in a column oven at 40 °C. The gradient  
12  
13 6 (solvent A, water/formic acid 980/20 v/v); solvent B, water-acetonitrile-formic acid  
14  
15 7 (80/900/20; v/v/v)) with a flow of 0.4 ml/min was: 0 – 1 min 30% B; 1-5 min linear  
16  
17 8 increase to 50% B; 5-5.1 min linear increase to 100%B; 5.1-7 min 100% B. Injection  
18  
19 9 volume was 40 µL. For 17β-19-nortestosterone the most abundant MRM transition  
20  
21 10 (used for the quantification) was acquired using the following conditions: precursor  
22  
23 11 ion mass (m/z) 275.1, Product ion mass (m/z) 109.2 with a collision energy of 25  
24  
25 12 (eV). A second MRM transition, used for the confirmation of the identity of 17β-19-  
26  
27 13 nortestosterone, was acquired using the following conditions: precursor ion mass  
28  
29 14 (m/z) 275.1, product ion mass (m/z) 145.1 with a collision energy of 20 (eV). For d<sub>3</sub>-  
30  
31 15 17βNT the most abundant MRM transition was acquired using the following  
32  
33 16 conditions: precursor ion mass (m/z) 278.2, product ion mass (m/z) 109.0 with a  
34  
35 17 collision energy of 30 (eV).  
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43 19 The concentrations of 17βNT in urine samples were estimated using the isotope  
44  
45 20 dilution method and a linear calibration curve were constructed for 17βNT by  
46  
47 21 fortifying blank urine aliquots at six different concentration levels (matrix-matched  
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49 22 standards, MMS).  
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## 54 24 **Results and discussion**

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1 The controlled treatment experiment was performed without problems, the animals  
2 were in good health and all animals were suitable for slaughter at the end of the  
3 experimental period. Including the spare animals we ended up with 6 treated barrows,  
4 6 control barrows and 5 boars for investigation.

5  
6 An overview of the live weights of the animals is listed in Table 1.

7 Mean live weight at slaughter was 121.6 kg for boars, 108.9 kg for barrows and 117.8  
8 kg for the 17 $\beta$ NT treated barrows. The treated barrows showed a significant ( $p < 0.05$ )  
9 better growth performance than the control barrows, and were not significantly  
10 different from the boars. At the facility where the animals were housed it appeared  
11 that the last weeks before slaughter there was an increased growth of the treated  
12 barrows as compared to the control animals (fig. 1).

13 **Insert Table 1 and Figure 1**

14  
15 These data confirmed our suggestion that 17 $\beta$ NT has a growth promoting effect in  
16 barrows. In literature the effects of anabolic steroids in pigs are not conclusive: In a  
17 series of experiments with implants in pigs, the combination of trenbolone and 17 $\beta$ -  
18 estradiol or 17 $\beta$ -estradiol and 17 $\beta$ -testosterone in barrows adversely affected growth  
19 rate and feed intake, but the implanted barrows had less fat than the controls (De  
20 Wilde & Lauwers, 1984). Other experiments with castrated males (Van Weerden &  
21 Grandadam, 1976) using implants with trenbolone and 17 $\beta$ -estradiol showed  
22 increased protein deposition and decreased fat deposition in the treated animals.  
23 Moreover the carcass weight was 4.5 kg higher than the controls, and carcass quality  
24 was improved and backfat thickness was reduced. In our experiment carcass

1 characteristics were not investigated because the aim of our experiment was to  
2  
3 examine the effects of  $17\beta$ NT on histology and residue levels in hair and urine.  
4  
5 At slaughter there appeared a striking difference in the weight and size of the  
6  
7 bulbourethral glands (Table 2), not only between barrows and boars, but also between  
8  
9 control and treated barrows. Treated barrows had larger and significant ( $p < 1 * 10^{-7}$ )  
10  
11 almost 3 times heavier bulbourethral glands than the controls (fig. 2). Increase of the  
12  
13 weight of male accessory glands is a well-known effect of androgens in castrated  
14  
15 males (Brandes, D, 1994; Booth, W.D., 1890). This increased size could be used as a  
16  
17 screening method for illegal treatment of barrows with androgenic hormones in the  
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19 slaughterhouse.  
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25 **Insert Table 2 and figure 2**

### 26 *Histology*

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30 Histological investigation of the testes of the boars revealed normal spermatogenesis  
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32 as was expected in these animals. The prostate showed abundant glandular tissue and  
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34 the bulbourethral glands showed mature glandular tissue (fig. 3) consisting of  
35  
36 mucinous acini with secretion of Alcian Blue positive (acidic) secretions. The barrows  
37  
38 showed atrophied glandular tissue in the prostate and bulbourethral gland showing  
39  
40 immature glandular tissue presenting with rosette shaped alveoli in abundant stroma  
41  
42 (fig. 4) and scarce PAS positive secretion. The treated barrows showed an increased  
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44 amount of glandular tissue in the prostate. The bulbourethral gland presented with  
45  
46 mature glandular tissue (fig. 5), which stained blue with Alcian Blue PAS staining.  
47  
48 This means that treatment with  $17\beta$ NT induced maturation of the glandular tissue of  
49  
50 the prostate and bulbourethral glands in barrows, causing histology resembling that of  
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52 boars. The results of the histological findings are in accordance with what Lauwers  
53  
54 (1984) described as androgen like changes, which he found in barrows treated with  
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1 allyltrenbolone. In treated barrows he observed development of the accessory glands  
2 in the physiological direction with more glandular tissue in the prostate and increased  
3 secretion in the bulbourethral gland and seminal vesicals. In barrows implanted with  
4 trenbolone acetate (López-Bote *et al.*, 1994), no changes in carcass weight were  
5 observed, but the implanted animals had less fat. They found enlargement of the  
6 prostate and bulbourethral gland with increased secretion and cyst formation. Cyst  
7 formation was not observed in our experiment, maybe because the animals were not  
8 continually exposed to hormones and the difference in androgenic and anabolic action  
9 between trenbolone and 17 $\beta$ NT.

10 **Insert figures 3-5**

11 *Residue analysis in urine*

12 All urines were analyzed for the presence of 17 $\beta$ NT. The urine was sampled at 4 time  
13 points, corresponding with day 12, 33, 61 and 81 of the trial. Levels of 17 $\beta$ NT were  
14 generally low (< 10  $\mu$ g/L) in barrows and treated barrows, except for one control  
15 barrow that had a level of 300  $\mu$ g/L. It was not clear why this animal had such a high  
16 level only at the last time point. No difference was observed between the 17 $\beta$ NT  
17 levels in the urine of the treated and control barrows. This may be due to the sampling  
18 schedule which was once a month and approximately two weeks after the day of  
19 treatment. In practice occasionally barrows and gilts are observed with high levels of  
20 urinary 17 $\beta$ NT, but possibly after treatment levels are only high in urine for a few  
21 days. In future experiments we have planned to investigate the kinetics of 17 $\beta$ NT in  
22 urine from hour to hour and from day to day after treatment. Another possibility is to  
23 examine the possible differences in metabolite profiles in treated and non-treated  
24 animals.

1 In boars high levels up to 400 µg/L of 17βNT were observed in urine, especially in  
2 older animals (table 3). Analysis of urine was in this trial not able to differentiate  
3 between treated and non-treated barrows, but most boars showed much higher levels  
4 than barrows.

5 **Insert table 3**

#### 6 *Residue analysis in hair*

7 In hair we measured the intact ester of 17βNT phenylpropionate. Hair was also  
8 sampled at 4 time points. For this method a calibration series was used from  
9 0 to 25 µg/kg. All values above this series are estimated (table 4).

10

11 In all non-treated barrows the levels were low <LLQ. All boars showed levels <LLQ.

12 In non-treated animals no esters were expected in the hair, these results confirmed this  
13 expectation. The treated animals showed from the start of the trial high levels 17βNT  
14 phenyl propionate in the hair, ranging from 15 to 220 µg/kg, which increased in time  
15 reaching a maximum level of 600 µg/kg (fig. 6).

16

17 We conclude that analysis of 17βNT phenyl propionate in hair is a good method to  
18 detect treated animals.

19 **Insert table 4 and figure 6**

20

#### 21 **Conclusion**

22 In this experiment we showed that 17βNT phenyl propionate is anabolic in barrows in  
23 the dosage used and that this treatment yields increased size and weight of the  
24 bulbourethral gland and profound histological alterations in the prostate en  
25 bulbourethral gland making them resemble the glands of boars. Inspection of the



1 weight and histology of bulbourethral glands could be used as a screening method for  
2  
3 illegal treatment of barrows with androgenic hormones in the slaughterhouse.  
4  
5 Treatment of barrows with 17 $\beta$ NT phenyl propionate can be detected by hair analysis  
6  
7 for the intact 17 $\beta$ NT esters. More investigation is needed to evaluate the excretion of  
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9 17 $\beta$ NT phenyl propionate in urine after treatment and the incorporation of 17 $\beta$ NT  
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11 phenyl propionate in hair after treatment in barrows as well as in gilts.  
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### 20 **Acknowledgement**

21 This project was financially supported by the Dutch Ministry of Economic Affairs,  
22  
23 Agriculture and Innovation.  
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1 **Table 1.** Body weights at slaughter

Body weight (kg)	Boar	Barrow	Barrow17β19NT
	116.4	103.2	113.4
	123.8	116.7	119.5
	119.2	114	120.1
	114.3	108.3	119.2
	134.1	114.1	122.5
		97.5	112.3
mean	121.6*	109.0	117.8**
s.d.	7.8	7.4	4.0

2

3 \* Boars significant heavier than barrows (p &lt;0.05)

4 \*\* Barrow17βNT significant heavier (p &lt;0.05) than control barrows

5 Weights barrow17βNT not significant different than boars

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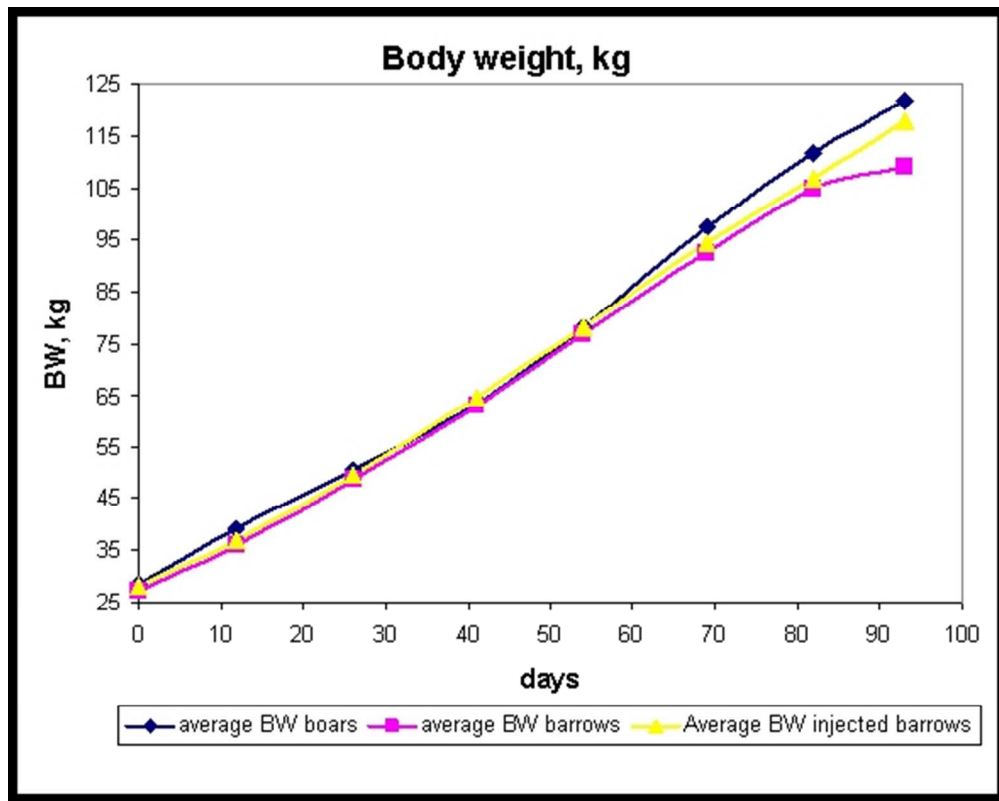


Figure 1. Growth of the animals during the trial  
156x124mm (96 x 96 DPI)

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**Table 2.** Weights of the bulbourethral glands

	<b>Bulbourethral gland 1 (grams)</b>	<b>Bulbourethral gland 2</b>
Control boar	69.9	65.5
Control boar	71.7	69.2
Control boar	84.4	90.7
Control boar	69.0	66.3
Control boar	75.7	70.6
<b>Mean</b>	<b>73.3** ± 8.1</b>	
Control barrow	*	*
Control barrow	4.1	3.9
Control barrow	2.3	3.3
Control barrow	3.6	3.4
Control barrow	3.3	2.8
Control barrow	3.9	3.2
<b>mean</b>	<b>3.4** ± 0.5</b>	
barrow17β19NT	9.5	10.5
barrow17β19NT	6.4	6.2
barrow17β19NT	9.6	10.3
barrow17β19NT	6.5	7.9
barrow17β19NT	7.0	8.2
barrow17β19NT	11.6	11.6
<b>mean</b>	<b>8.8** ± 2.0</b>	

\* not available

\*\* means of the paired organs

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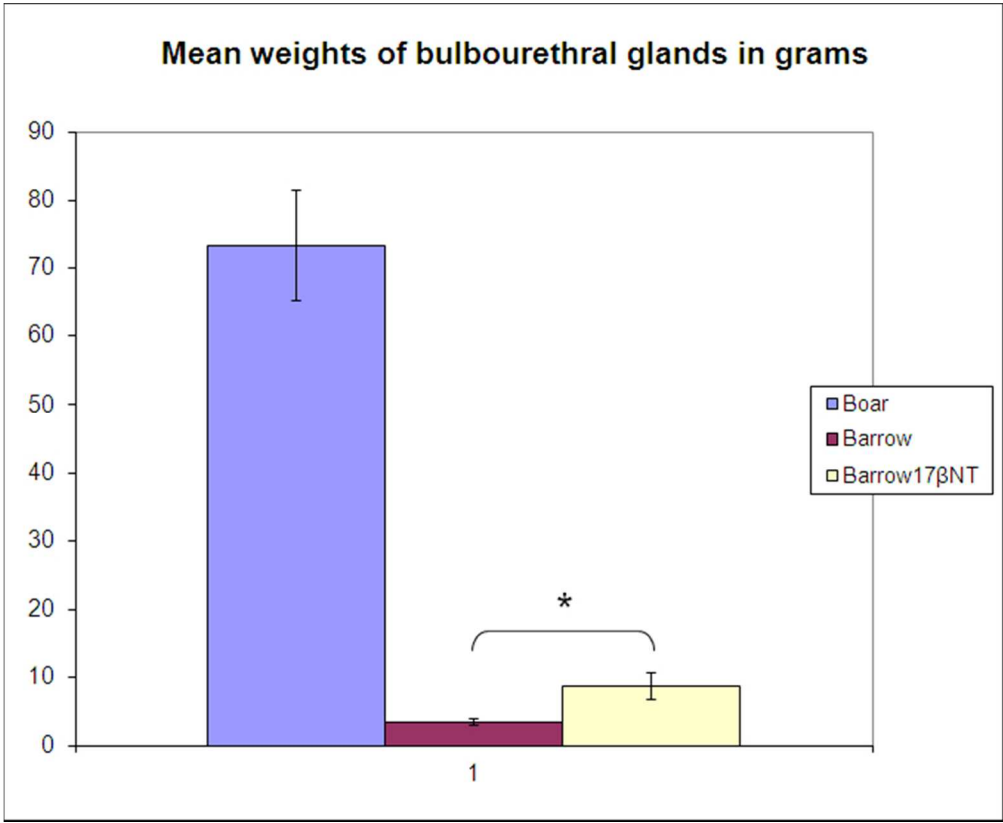


Figure 2: Mean weights of the bulbourethral glands

**Table 3.** Levels of 17 $\beta$ -19-nortestosterone in urine per animal

Concentration 17 $\beta$ -19-nortestosterone ( $\mu\text{g/L}$ )  
(LLQ = 0.5  $\mu\text{g/L}$  for pig urine)

Animal number	Sex/ treatment	Time point 1 (prior to treatment)	Time point 2	Time point 3	Time point 4
1087	Barrow	35	1	5	300
6504	Barrow	2	2	8	3
7630	Barrow	n.d.*	1	20	10
7702	Barrow	n.d.	1	13	7
9975	Barrow	n.d.	3	5	10
9950	Barrow	n.d.	2	6	No sample
9373	Barrow+**	12	2	4	4
8111	Barrow+	n.d.	1	2	3
5133	Barrow+	n.d.	0.5	1	3
6185	Barrow +	n.d.	2	4	5
6559	Barrow+	n.d.	2	1	5
7177	Barrow+	7	3	3	2
1210	Boar	15	100	125	175
4473	Boar	185	165	150	2
7805	Boar	-	130	120	40
8775	Boar	55	140	130	100
9631	Boar	50	80	190	400

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\* n.d. = not detected

\*\* barrow+ = barrow17β19NT

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1 **Table 4.** Levels of 17 $\beta$ -19-nortestosterone phenylpropionate in hair per animal

Concentration 17 $\beta$ -19nortestosterone phenylpropionate  
( $\mu\text{g}/\text{kg}$ ), (LLQ = 2.5  $\mu\text{g}/\text{kg}$  for pig hair)

Animal number	treatment	Time point 1 Prior or treatment	Time point 2	Time point 3	Time point 4
1087	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
6504	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
7630	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
7702	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
9950	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
9975	Barrow	< LLQ	< LLQ	< LLQ	< LLQ
8111	Barrow+*	< LLQ	17	240	330
9373	Barrow+	< LLQ	220	15	340
5133	Barrow+	< LLQ	15	60	200
6185	Barrow+	< LLQ	365	125	600
6559	Barrow+	< LLQ	13	175	265
7177	Barrow+	< LLQ	120	20	90
7805	Boar	< LLQ	< LLQ	< LLQ	< LLQ
8775	Boar	< LLQ	< LLQ	< LLQ	< LLQ
9631	Boar	< LLQ	< LLQ	< LLQ	< LLQ
1210	Boar	< LLQ	< LLQ	< LLQ	< LLQ
4473	Boar	< LLQ	< LLQ	< LLQ	< LLQ

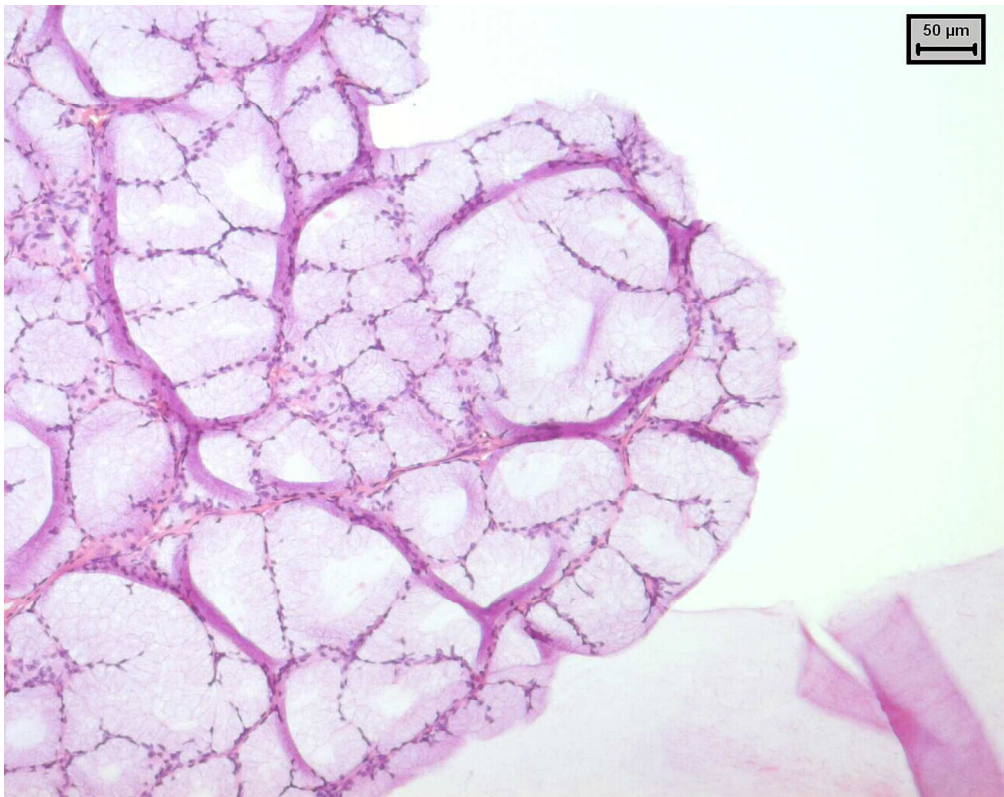
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1       \*barrow+ = barrow17β19NT

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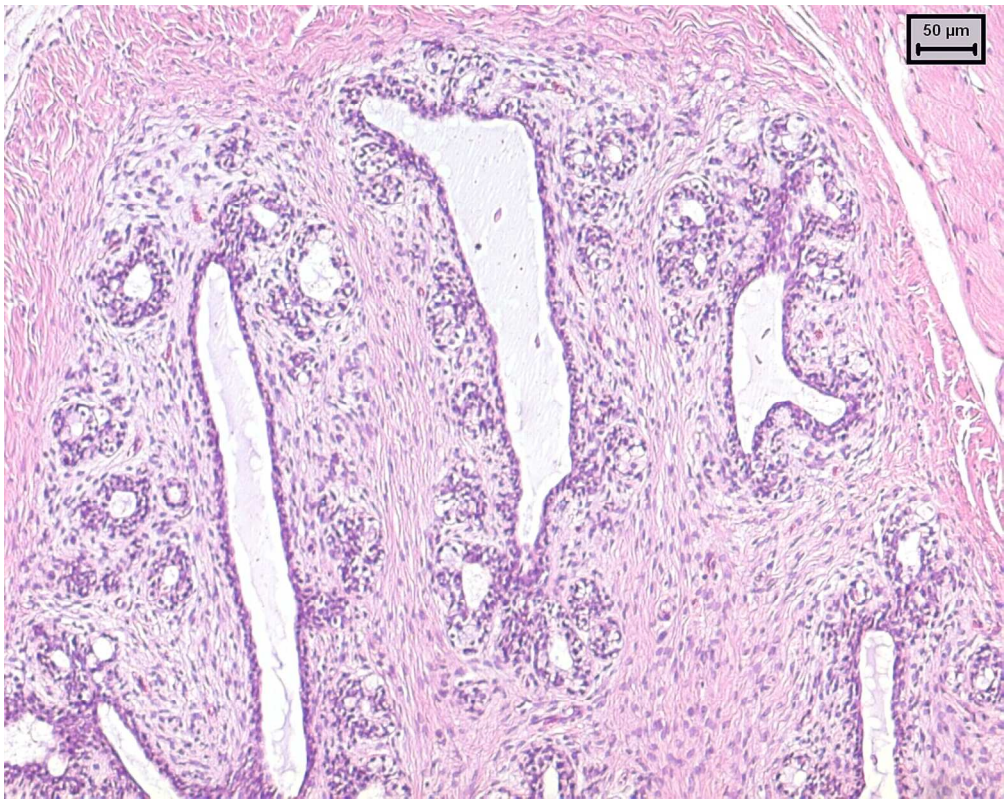
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Bulbourethral gland boar (HE), showing mature glandular tissue  
343x272mm (96 x 96 DPI)

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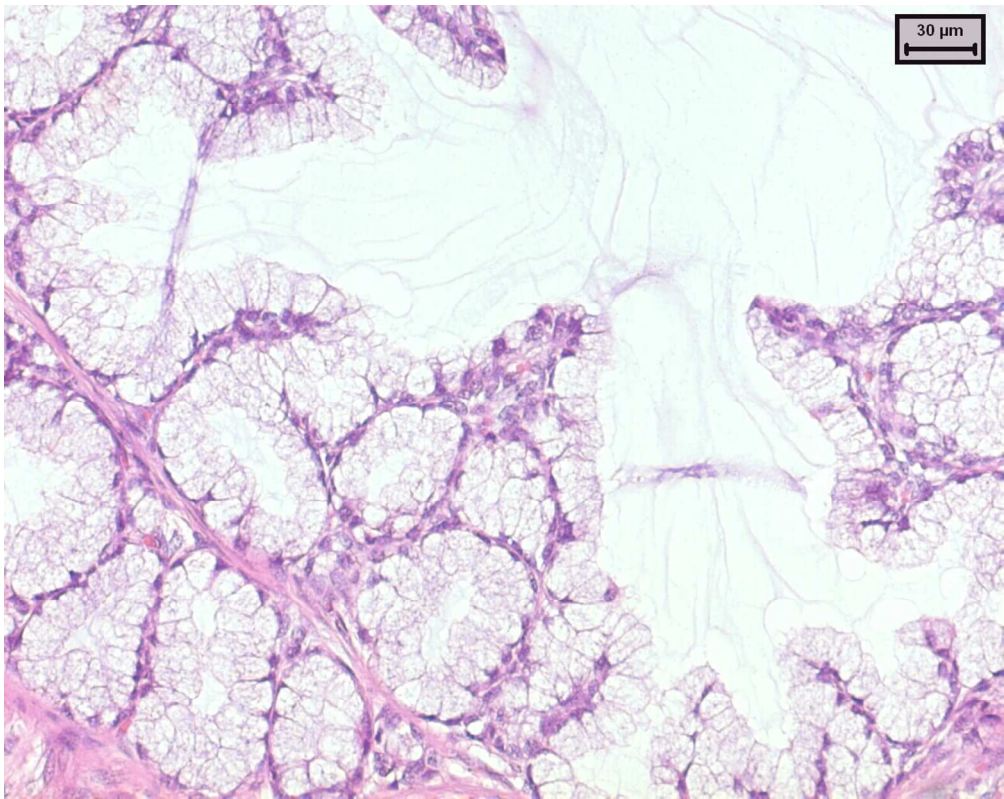


Bulbourethral gland barrow, showing immature glandular tissue (HE)  
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Bulbourethral gland of a treated barrow, showing mature glandular tissue (HE).  
343x272mm (96 x 96 DPI)

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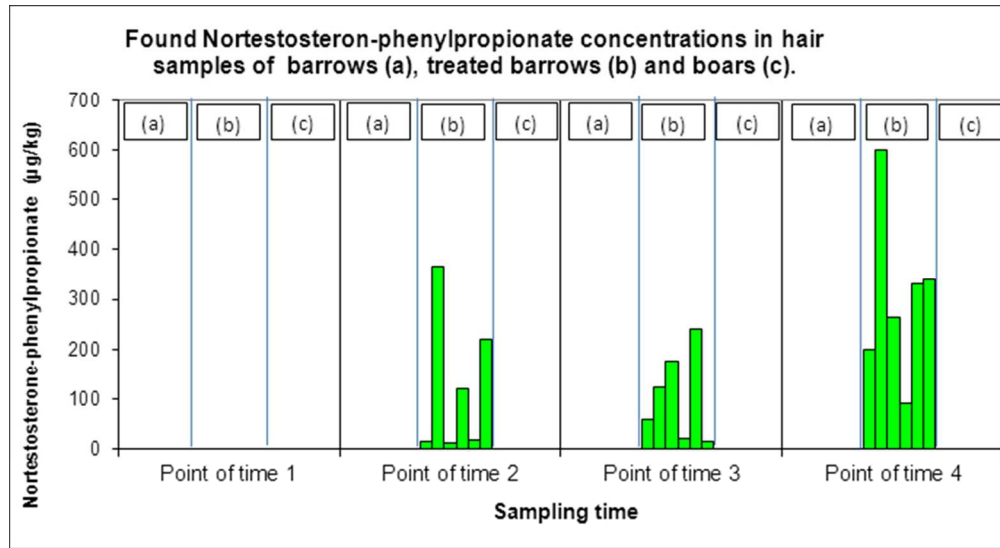


Figure 6. 17βNT phenylpropionate in hair  
181x98mm (96 x 96 DPI)

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