Histomorphological and immunohistochemical findings in testes, bulbourethral glands and brain of immunologically castrated male piglets

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Abstract

The aim of this study was the histological and immunohistochemical evaluation and comparison of testicular, bulbourethral and brain tissue in immunized and intact control boars. Fourteen male piglets, aged between 10 and 16 weeks, were vaccinated twice subcutaneously 4 to 5 weeks apart with Improvac®, an anti-GnRH vaccine. The pigs were sacrificed 1 to 16 weeks following the second injection. Testicular weight was recorded and various tissue samples were collected and fixed in formalin and Bouin's fixative for histological examination. In addition, 2 boars were immunized five times and slaughtered 60 weeks after the last injection. Histological and immunohistological studies performed on testes and epididymes showed clear signs of atrophy in the immunized animals and a significant reduction in paired testes weight was seen in treated boars. Microscopically, the mean diameter of the seminiferous tubules was markedly reduced. Spermatogonia as well as few spermatocytes were visible between the Sertoli cells and Leydig cells were atrophic. None or only few spermatozoa were detected in the epididymis. The bulbourethral glands of immunocastrated pigs were smaller than in control pigs and showed histological evidence of atrophy. Immunohistological detection of LH and FSH in the pituitary gland of treated and control boars showed no quantifiable difference in the amount of these two gonadotropins and no lesions were visible in the hypothalamus and the pituitary gland. From our findings it can be concluded that the anti-GnRH vaccine Improvac® induces severe atrophy of testes and bulbourethral glands in immunized pigs. This effect appears to be reversible, depending on the immune response of each animal and the time elapsed after the last booster injection.

Histomorphologische und immunhistochemische Befunde von Hoden, Bulbourethraldrüsen und Gehirn bei immunologisch kastrierten männlichen Ferkeln

Ziel dieser Arbeit war die histologische und immunhistochemische Auswertung sowie der Vergleich von Hoden, Bulbourethraldrüsen und Gehirngewebe zwischen immunologisch kastrierten und unbehandelten Ebern. Vierzehn männliche Ferkel, 10 bis 16 Wochen alt, wurden zweimal im Abstand von 4 bis 5 Wochen subkutan mit Improvac®, einer anti-GnRH Vakzine, behandelt. Die Eber wurden 1 bis 16 Wochen nach der zweiten Injektion geschlachtet. Von allen Tieren wurde das Hodengewicht bestimmt und zur histologischen Untersuchung von verschiedenen Organen Gewebsproben entnommen und in Formalin oder Bouin fixiert. Zwei zusätzliche Tiere wurden fünfmal immunisiert und erst 60 Wochen nach der letzten Injektion geschlachtet. Die histologische und immunhistologische Auswertung von Hoden und Nebenhoden zeigte klare Anzeichen von Atrophie. Das Hodengewicht der immunisierten Tiere war signifikant erniedrigt. Mikroskopisch war der durchschnittliche Durchmesser der Tubuli seminiferi stark reduziert und zwischen den Sertolizellen waren Spermatogonien sowie wenige Spermatozyten sichtbar. Die Leydigzellen waren atrophisch. Im Nebenhoden konnten keine oder nur vereinzelt Spermatozoen gefunden werden. Die Bulbourethraldrüsen immunokastrierter Schweine waren kleiner als diejenigen der Kontrolltiere und zeigten Anzeichen einer Atrophie. Der immunhistologische Nachweis von LH und FSH in der Hypophyse behandelter und unbehandelter Schweine wies keinen quantifizierbaren Unterschied in der Menge beider Gonadotropine auf und im Hypothalamus und der Hypophyse waren keine Läsionen erkennbar. Unsere Untersuchungen zeigen, dass die anti-GnRH Vakzine Improvac® bei immunisierten Schweinen eine starke Atrophie von Hoden und Bulbourethraldrüsen verursacht. Dieser Effekt scheint je nach individueller Immu-

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nitätslage und Zeitspanne nach der letzten Booster-Injektion reversibel zu sein.

Schlüsselwörter: Schwein, Hoden, immunologische Kastration, Immunhistochemie, Gehirn, Hypophyse

Introduction

Surgical castration of male piglets without anesthesia has been a subject of controversy in Europe during the last years mainly because of animal welfare. In order to eliminate boar taint without compromising the anabolic effect, immunization against endogenous GnRH several weeks before slaughter has been investigated by many researchers world-wide (English et al., 1983; Awoniyi et al. 1988; Bonneau et al., 1994; Thompson, 2000; Dunshea et al., 2001; Metz et al., 2002; Turkstra et al., 2002; Zeng et al., 2002; Jaros et al., 2005). Anti-GnRH vaccines have been shown to induce antibodies, which will bind endogenous GnRH within the hypothalamic-pituitary portal vessels and prevent it from binding to receptors on pituitary gonadotrophes. Hence, the secretion of both gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is stopped and the production of androgens, as testosterone and androstenone inhibited. Overall, testosterone and androstenone concentrations are suppressed to similar levels of surgically castrated animals. As a consequence both spermatogenesis and steroidogenesis are inhibited and the testes weight reduced (English et al., 1983; Awoniyi et al., 1988; Oonk et al., 1995a; Zeng et al., 2002; Jaros et al., 2005).

Specific cells in the testes can be visualized by immunohistological markers. Amselgruber et al. (1994) described the S100 immunoreactivity of Sertoli cells and found that S100 is also expressed in other testicular structures like Leydig cells but in a species-specific manner. In pigs, Sertoli cells show a strong immunoreactivity whereas Leydig cells and endothelial cells react only weakly. Leydig cells are the primary location of ferritin and iron storage in the testes and hence they can be stained by ferritin antibodies (Wise et al., 2003). The objective of this study was to examine the testes, the bulbourethral glands and the brain of male pigs using histological and immunohistological techniques after immunization against GnRH.

Animals, Materials and Methods

Animals

A total of 20 male pigs (100% Swiss Large White) were used for our experiment. The animals were housed in a half-open pig pen, fed twice daily with standard fattening food. Water was given ad libitum. Four adult boars between 6 and 12 months served as controls.

Experimental design

Immunization was performed with Improvac® (Pfizer Animal Health), which consists of a modified synthetic gonadotropin-releasing hormone (GnRH) bound to a carrier protein and an aqueous adjuvant (CSL Technical Update; Jaros et al., 2005). Sixteen pigs were immunized twice subcutaneously beginning between 10 to 16 weeks of age with a booster injection 4 to 5 weeks later. In 2 animals three further booster injections were given at 2 week intervals. Fourteen pigs were sacrificed from 1 to 16 weeks after the second injection. The remaining 2 pigs were used for collecting hyperimmune serum and sacrificed 60 weeks after the fifth injection.

During necropsy paired testes weight was determined and the length and diameter of the bulbourethral glands measured. Testes were cut in half and a transverse section was collected from the center. Pieces from the epididymes (head and tail) were also collected and fixed together with testicular tissue in formalin and Bouin's fixative. Apart from testes, the bulbourethral glands as well as the pituitary gland (neuro- and adenohypophysis) and coronal sections from the brain (cortex, basal ganglia, gyrus dentatus, hypothalamus, thalamus, mesencephalon, cerebellum and medulla oblongata) were also histologically evaluated. Furthermore, major endocrine (pancreas, thyroid gland, adrenal gland) and parenchymatous organs (lung, heart, liver, kidney, spleen, intestines) were examined as well.

Histology

The testes and epididymes fixed in Bouin's fixative were thoroughly washed in water after 18 hours fixation and afterwards stored in 4% formalin. All other samples were fixed in 4% formalin and were than paraffin-embedded. Slides of these organs were stained with hemalaun-eosin, examined and evaluated microscopically. For morphometry the diameter of the tubuli seminiferi contorti of the testes fixed in Bouin's were measured with the program AxioVision 2.05. Images were captured with an AxioCam 3.0 camera (Carl Zeiss) on top of an Olympus AH-2 microscope (magnification 25 times). The diameter (cross sections) of 10 randomly selected tubuli seminiferi contorti of the central testicular region from the left and right testicle was measured and mean values established.

Immunohistology

All antibodies (ferritin, S100, LH and FSH) were employed on paraffin-embedded sections. The formalin fixed paraffin-embedded testicular tissue samples were deparaffinized, rehydrated through graded alcohol and washed in water followed by counterstaining in hemalaun for 2 to 3 min. For each of the immunohistochemical assays, a positive and negative control were included. During each step the slides were meticulously washed with PBS.

Ferritin-Immunohistochemistry (EnVision™ method)

Ferritin was used as a marker for Leydig cells. Endogenous peroxidase was inhibited with 3% H_2O_2 (3% H_2O_2 with 0,2% NaN_3 (sodiumazide) in water), subsequently a protein block (DAKO® Protein Block Serum-Free, Ready-to-Use, Code No. X0909, DAKO, Zug, Switzerland) was performed. Both steps were carried out for 10 min. at room temperature (RT). Incubation with ferritin at RT overnight (ferritin rabbit anti-human, Code No. A 0133, DAKO®) was conducted at a dilution of 1:200. As a secondary antibody the anti-rabbit EnVision (EnVision™, Code No. K4003, DAKO®) was added for 30 min. at RT. AEC (3-Amino-9-ethyl Carbazole Substrate Kit, Zymed® Laboratories Inc, 00-2007, San Francisco) was used as indicator.

S100-Immunohistochemistry (ChemMate™ method)

S-100 was used as a marker for Sertoli cells. Slides used for S100 (polyclonal rabbit anti-S100, ready-to-use, Code No.H0066, DAKO®) immunhistochemistry were boiled in the microwave with citrate buffer (S2031, DAKO®) for 30 min. for antigen retrieval and thereafter the endogenous peroxidase was blocked (Peroxidase Blocking Reagent, S2001, DAKO®) for 5 min. at RT. Afterwards the ChemMate kit was applied (ChemMate™, Detection Kit, rabbit/mouse, Code No. K5003, Peroxidase, DAKO®) as described by the manufacturer. As chromogen AEC was applied.

LH- and FSH-Immunohistochemistry (EnVision™ method)

The pituitary gland slides were incubated for 10 min. at RT with 3% H_2O_2 (3% H_2O_2 with 0,2% NaN_3 (sodiumazide) in water) to block the endogenous peroxidase. Afterwards, the primary LH (rabbit anti-human LH, Code No. N1543, DAKO®) and FSH antibodies (rabbit anti-human FSH, Code No. N1539, DAKO®) were applied undiluted and incubated for 90 min. at 37° C. An anti-rabbit (Code No. K4003, DAKO®) secondary antibody was used for 30 min. at RT. Positive reaction was observed under the microscope with AEC.

Statistical analysis

Data were analyzed using StatView 5.0 software program (SAS Institute Switzerland) and ANOVA was performed. The age of the boars was considered as covariate in the model. All data are presented as mean ± standard deviation (SD). Statistical significance was based on $P < 0.05$.

Results

Testicular weight and morphology

Table 1 summarizes the number of animals and injections as well as the mean testicular weight, the mean diameter of tubuli seminiferi and the mean length and diameter of the bulbourethral gland. With the exception of one animal (no. 10) which was excluded from further analysis mean (± SD) testicular weight was significantly ($P < 0.0001$) reduced in all vaccinated pigs (89.9 ± 55.1 g) compared to control animals (633.3 ± 76.4 g). Histologically a significant ($P = 0.0041$) diminution of the mean (± SD) diameter of the tubuli seminiferi has been observed in treated compared to control pigs (96.2 ± 32.8 µm vs. 259.7 ± 13.7 µm). These data were also analyzed regarding age and animal as well as interactions between age and animal. For the testicular weight the calculated P-values were: age $P = 0.0020$, animal $P < 0.0001$ and the interaction between age and animal $P = 0.2191$ and for the diameter of the tubuli seminiferi: age $P = 0.0600$, animal $P = 0.0041$ and the interaction between age and animal $P = 0.3175$.

In normal testicular tissue the spermatogenesis was fully developed from spermatogonia up to spermatozoa (Fig. 1). In all immunocastrated animals the

Table 1: Animals, number of injections, slaughter time after last injection, testes weight, diameter of tubuli seminiferi as well as length and diameter of bulbourethral gland in control and immunized male pigs.

Animal	Age[1] at slaughter	Number of injections	Slaughter time after last injection (weeks)	Paired testes weight (g)	Diameter of tubuli seminiferi (µm)		Bulbourethral gland (cm)	
					Mean[2]	Range	Length[3]	Diameter[3]
Control	6 mo	none	n. d.	550	247	206–289	14.0	3.0
Control	6 mo	none	n. d.	650	249	204–293	18.0	4.0
Control	12 mo	none	n. d.	700	269	220–318	17.0	4.0
Control	12 mo	none	n. d.	800	274	228–319	19.0	5.0
No. 1	14 w	2	1	82	109	100–143	6.2	1.2
No. 2	15 w	2	1	48	99	64–105	5.2	1.1
No. 3	16 w	2	2	32	57	46– 67	3.5	1.1
No. 4	17 w	2	3	43	63	49 – 76	5.2	1.0
No. 5	20 w	2	4	47	118	78– 74	6.1	0.5
No. 6	22 w	2	4	49	58	49– 63	5.0	1.0
No. 7	20 w	2	4	76	63	47– 74	5.6	0.6
No. 8	19 w	2	5	64	68	55– 78	4.7	0.8
No. 9	20 w	2	7	83	103	88–131	5.1	1.0
No. 10	24 w	2	7	710[a]	146	118–204	n. d.	n. d.
No. 11	24 w	2	8	240	121	112–176	8.7	1.2
No. 12	25 w	2	9	112	163	134–206	7.0	1.0
No. 13	28 w	2	12	112	99	87–115	7.6	2.0
No. 14	32 w	2	16	135	130	110–146	7.2	1.9
No. 15	24 mo	5	60	136	119	100–132	n. d.	n. d.
No. 16	24 mo	5	60	553[b]	280	226–347	14.0	5.5

[1]mo: months; w: weeks; n. d.: not determined; [2]mean of 10 tubuli seminiferi of each testicle; [3]mean of left and right gland
[a]poor responder (excluded from further analysis); [b]regeneration

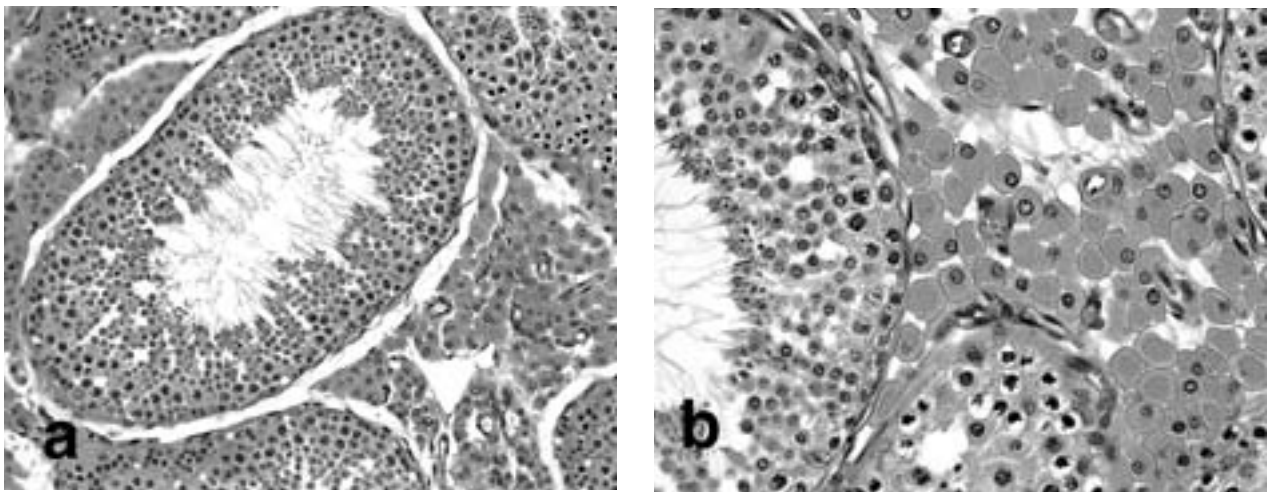

Figure 1: Testicular tissue of a control boar (6 mo) with normal tubuli seminiferi, regular spermatogenesis and prominent Leydig cells (1a, x 20; 1b x 40). HE.

majority of cells visible in the tubuli seminiferi were Sertoli cells and spermatogonia. The differentiation or classification of primary and/or secondary spermatocytes was not possible. In some animals, mostly in those sacrificed late after the booster injection, only few spermatocytes and spermatids were discernible

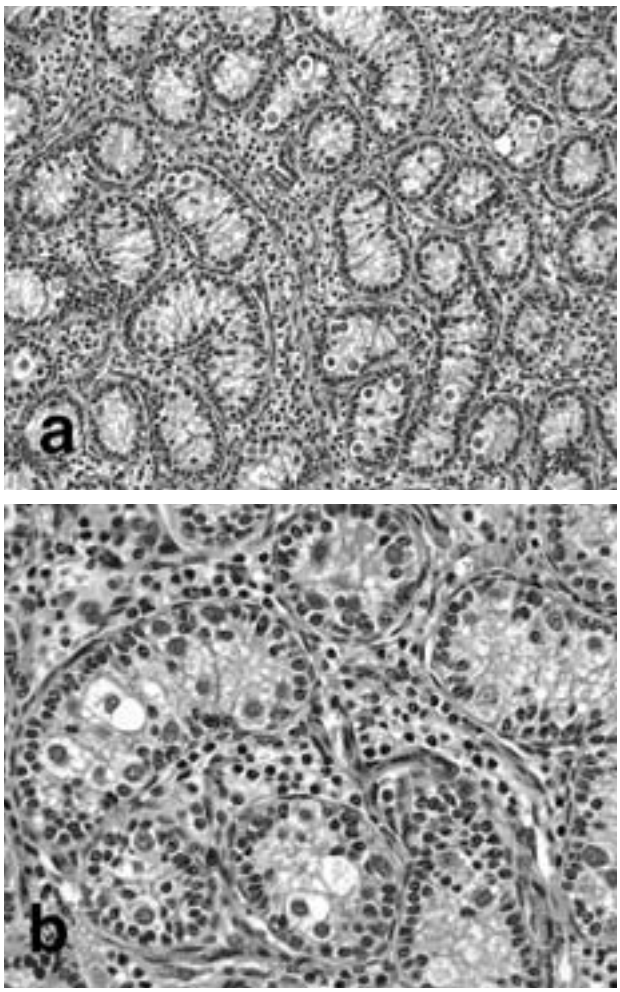

Figure 2: Testicular tissue of boar No. 3, two weeks (2a, x 20) and boar No. 4, three weeks (2b, x 40) after the second injection. Markedly reduced diameter of tubuli seminiferi with only spermatogonia and prominent Sertoli cells. Leydig cells are reduced in size. HE.

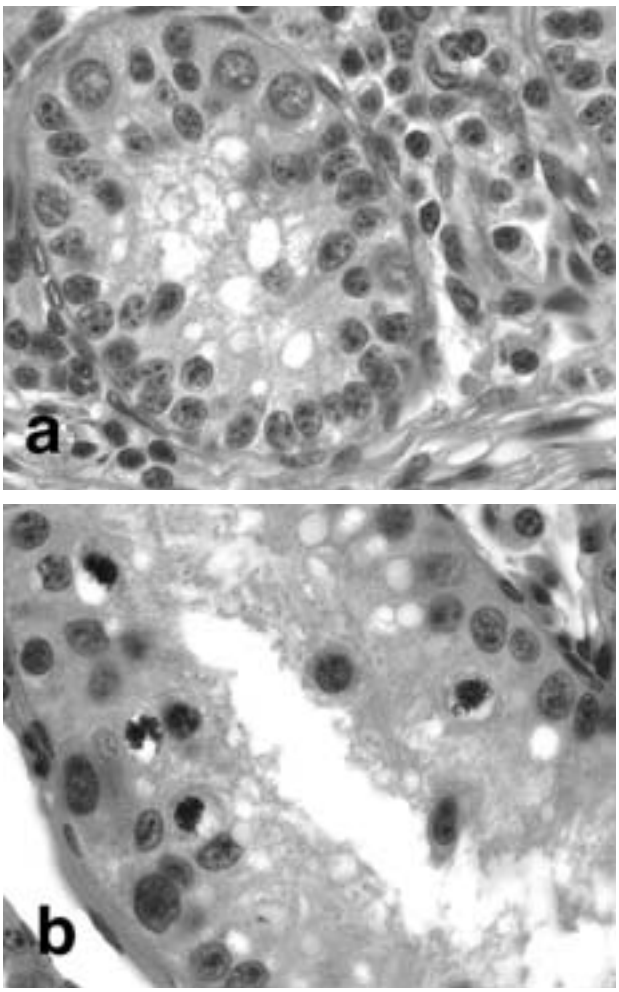

Figure 4: Testicular tissue of boar No. 4, three weeks after the second injection. Prominent Sertoli cells (4a, x 100) and few large spermatogonia with mitotic figures (4b, x 100) can be seen. HE.

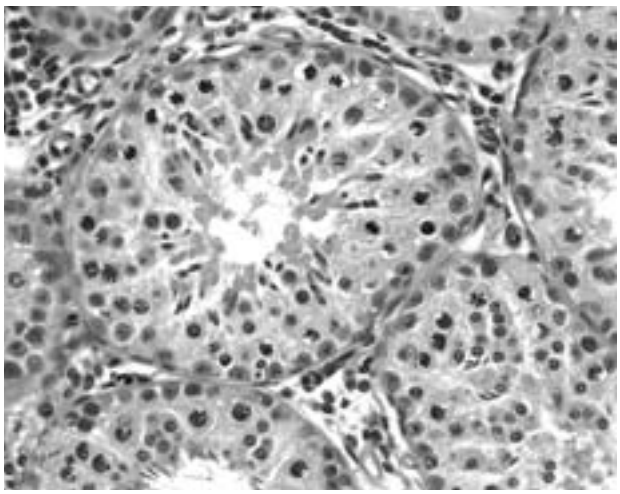

Figure 3: Testicular tissue of boar No. 14, sixteen weeks after the second injection. Sertoli cells, spermatogonia, spermatocytes and spermatids are discernible. Leydig cells are reduced in size (x 40). HE.

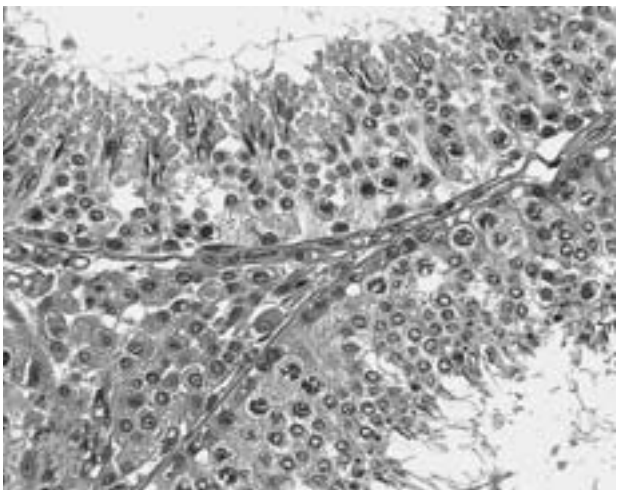

Figure 5: Testicular tissue of boar No. 16, sixty weeks after the fifth injection. Regenerated testicular spermatogenesis with well visible Leydig cells (x 40). HE.

(Fig. 2 and 3). The epididymal head and tail of treated animals showed no or only few spermatozoa in contrast to control pigs that had a very large number of spermatozoa in the epididymis. In the process of regeneration after the booster injections spermatogonia with or without mitotic figures and none or

only few spermatocytes and spermatids were visible (Fig. 4). One of the 2 pigs immunized five times (No. 16) had fully regenerated testicular tissue with normal spermatogenesis and prominent Leydig cells 60 weeks after the last injection (Fig. 5). The mean diameter of tubuli seminiferi was 280 µm.

Ferritin and S100 immunohistology and testicular histology

In the immunocastrated animals the tubuli seminiferi were lined mainly by spermatogonia and Sertoli cells as made visible with S100 immunohistochemistry (Fig. 6). Leydig cells of control boars were large, polygonal, had a prominent nucleus and nucleolus and large amounts of homogenous eosinophilic cytoplasm. In immunocastrated animals the Leydig cells appeared shrunken and small. Because they were difficult to discern in histology, visualization by ferritin was employed (Fig. 7). In regenerating testicular tissue, Leydig cells were easily visible, large and prominent and similar in amount as in intact boars.

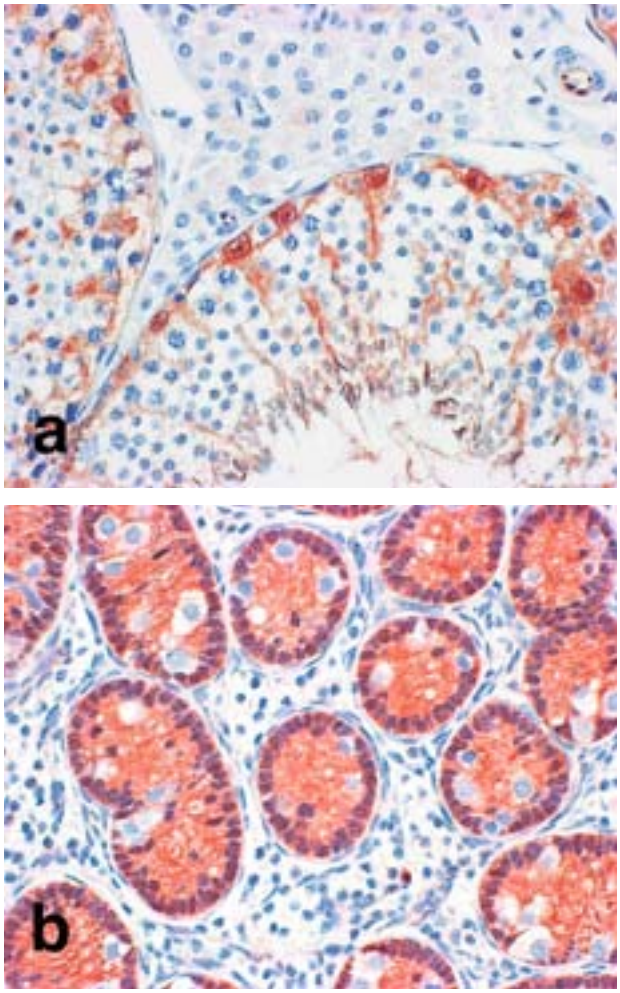

Figure 6: Testicular tissue of a control boar (6a, x 40) and of boar No. 3, two weeks after the second injection (6b, x 40). Immunohistochemical staining for S100 demonstrating numerous positive Sertoli cells. Because of diminished spermatogenesis (6b) the Sertoli cells are prominently labeled with S-100 and overlap the whole lumen of the tubuli seminiferi (ChemMate-method).

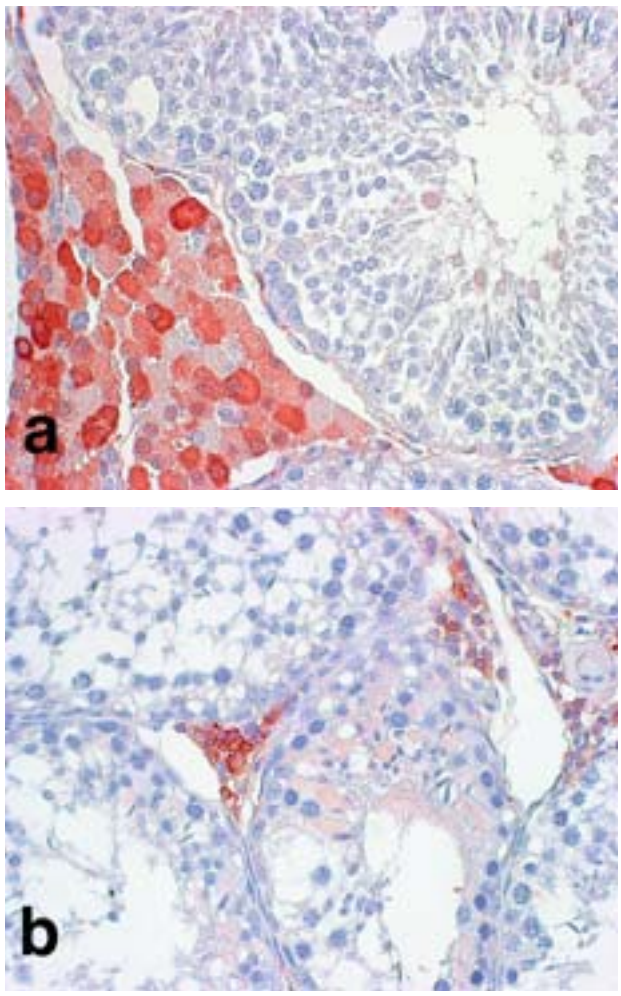

Figure 7: Testicular tissue of a control boar (7a, x 40) and of boar No. 12, nine weeks after the second injection (7b, x 40). Immunohistochemical staining for ferritin demonstrating positive Leydig cells. In the immunized boar (7b), the quantity of Leydig cells is substantially diminished (EnVision-method).

Bulbourethral gland measurement and morphology

In controls, mean (± SD) length of the bulbourethral glands was 17.0 ± 2.1 cm as compared to 5.9 ± 1.4 cm in immunocastrated animals ($P < 0.0006$) and regarding the mean (± SD) diameter, values were 4.0 ± 0.8 cm vs. 1.1 ± 0.4 cm ($P = 0.0114$). The bulbourethral gland of boar with regenerating testicular tissue 60 weeks after the fifth injection (no. 16) had a mean diameter of 5.5 cm and was 14 cm long. Histologically the gland lobes of immunocastrated pigs were atrophic.

Brain and pituitary gland: histology and LH- and FSH-immunohistology

In the brain and pituitary gland (Fig. 8) no inflammatory or degenerative processes were seen. LH- and FSH-positive cells (Fig. 9 and 10) were mostly distributed diffusely in the pars distalis (adenohypophysis) and aggregated near the region where the

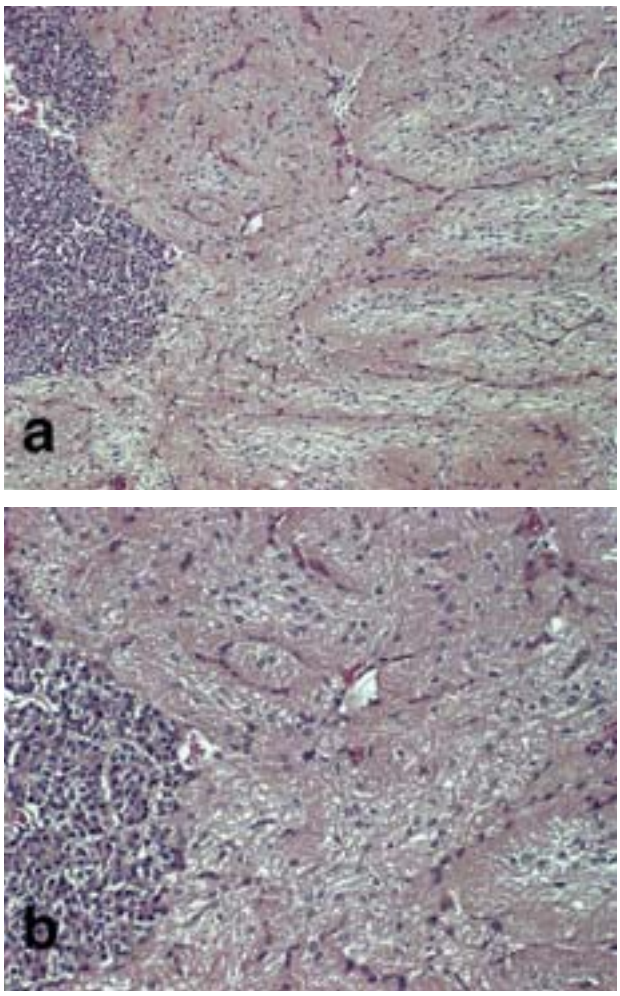

Figure 8: Neurohypophysis (pars nervosa) and part of adenohypophysis (pars distalis) of boar No. 6, four weeks after the second injection. No inflammatory or degenerative changes are visible (8a, x 10; 8b, x 20). HE.

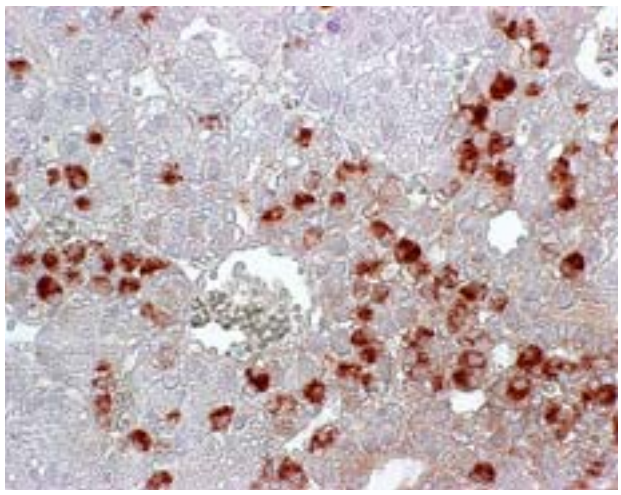

Figure 9: Pituitary gland of boar No. 6, four weeks after the second injection. Immunohistochemically LH-positive cells are diffusely distributed in the pars distalis of the pituitary gland (x 40). EnVision-method.

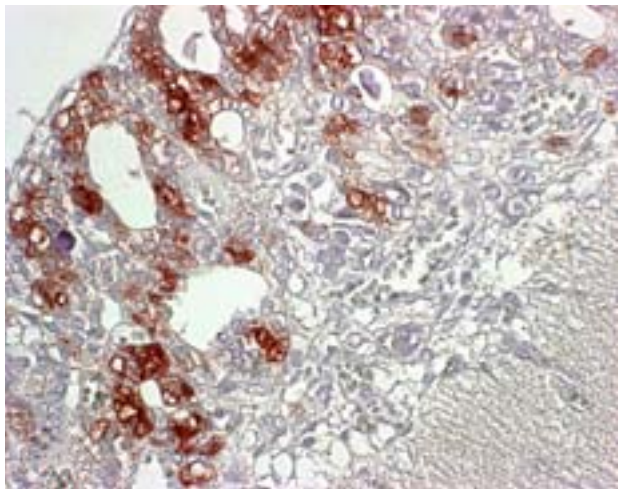

Figure 10: Pituitary gland of boar No. 2, one week after the second injection. Immunohistochemically FSH-positive cells are aggregated in the pars distalis adjacent to the pars nervosa of the pituitary gland (x 40). EnVision-method.

pars nervosa is adjacent to the pars distalis in pigs. No quantifiable distinction in number and staining intensity could be made between the amount of positive LH- and FSH-cells in immunized compared to control pigs.

Discussion

Immunological castration using anti-GnRH vaccines is an effective alternative to surgical castration in male animals of different species for reducing undesirable male-related characteristics, like boar-taint, sexual behavior and aggression (English et al., 1983; Awoniyi et al., 1988; Esbenshade et al., 1990; Meloen et al., 1994; Cook et al., 2000; Thompson, 2000; Turkstra et al., 2002; Janett et al., 2003; Clement et al. 2005; Jaros et al., 2005; Turkstra et al., 2005, Burger et al., 2006). The main focus of this study was the morphological characterization of testes, bulbourethral glands and brain sections using histology and immunohistochemistry in male pigs after immunization with a GnRH vaccine (Improvac®) and to compare these results with untreated animals.

S100 and ferritin are two immunohistological markers that are known to be expressed in the pig in the Sertoli and Leydig cells respectively (Amselgruber et al., 1994; Wise et al., 2003). Thus, it was possible to differentiate more clearly between these cells and the cells implicated in spermatogenesis. The testes of immunocastrated boars showed mostly spermatogonia with or without mitotic figures and only few or no spermatocytes and spermatids depending on the time elapsed between the last booster injection and euthanasia. Leydig cells appeared smaller in immunocastrated boars but it was not possible to evaluate if they were also numerically reduced. Awoniyi et al. (1988) also found multinucleated giant cells in the tubuli seminiferi of the testes and disrupted Sertoli cells, which they interpreted as degeneration. In our

study, these features were not visible at any time point after the second booster injection not even 1 year after four booster injections.

In our study no lesions were seen macroscopically or microscopically in major endocrine (pancreas, thyroid gland, adrenal gland) and parenchymatous organs (lung, heart, liver, kidney, spleen and intestine) of immunocastrated boars. Furthermore, no microscopical lesions could be found neither in the hypothalamus, (and all the examined brain regions) nor in the pituitary gland of vaccinated boars. Our results do not support those of Molenaar et al. (1993) who described various degrees of inflammatory reactions as well as dystrophy of perikarya, particularly in the median eminence. We believe that the discrepancy between both studies can most likely be explained by the different types of antigen and adjuvants used, which may elicit specific immunological reactions at sites of GnRH accumulation outside the blood-brain barrier. However, in the study by Molenaar et al. (1993) not only immunized animals but also sham-vaccinated control animals had brain abnormalities related to various diseases which were found at post mortem examination. Therefore, another study (Oonk et al., 1995b) was designed at investigating the effects of a GnRH-tandem vaccine on brain morphology using specific-pathogen-free (SPF) pigs. Extensive evaluation showed no abnormalities in brain histology supporting the results in this study. Whether differences in age at first vaccination (6 weeks) may also have contributed to the deviating findings remains speculation. Surprisingly enough, in the present experiment pituitary lesions could never be found, even 8 to 16 weeks after the second immunization and as shown in boar number 15, which was vaccinated 5 times, not even 60 weeks after the last injection.

No distinct variation in the amount and labelling intensity of LH- and FSH-positive cells in the adenohypophysis (pars distalis) of immunized and control pigs could be seen. Correct interpretation was sometimes difficult because the plane and direction of the cut section through the pituitary gland was difficult to standardize. Our results agree with those of Molenaar et al. (1993) who also found that LH-producing cells in the adenohypophysis and the pars tuberalis were not markedly different in number, size or stainability from those in the control animals. Although antibodies against GnRH, LH or FSH were not measured in this study, it has been shown that antibodies against GnRH bind endogenous GnRH and prevent it from binding to receptors on pituitary gonadotrophs (Thomson, 2000). This interpretation is also sustained by findings of Turkstra et al. (2002) who described that serum LH was dramatically reduced in immunocastrated boars. Wagner and Claus (2004) also reported a decrease of LH and testosterone but not of FSH in pig blood plasma after vaccination with Improvac®.

One animal (No. 10) from which only the testes were available for examination, showed no clear vaccination effect even 7 weeks after the second injection. The testes of this animal weighed 710 g and histology displayed normal spermatogenesis. On the basis of these results this animal could be termed a non-responder as described in the literature (Zeng et al., 2002). In a recent study (Jaros et al., 2005) using 270 pigs immunized with Improvac®, only 2 (< 1%) animals had high androstenone levels and normal testicular weights, confirming that antibody response can vary between individual animals. From the 2 boars which were vaccinated five times one of them (No. 15) had small testes (136 g) while the other one (No. 16) showed a transient decrease in testicular size but when slaughtered 60 weeks after the fifth injection the testes had regenerated (553 g). Spermatogenesis was fully developed and the mean diameter of the tubuli seminiferi was 280 μm and Leydig cells were prominent and easily visible on histological examination. Therefore, testicular tissue seems to have a potential for regeneration even after several booster injections. Fertility of this boar was confirmed by successful mating with a sow. Regeneration of reproductive function after GnRH vaccination has also been reported in mares (Imboden et al., in press) and stallions (Clement et al., 2005; Burger et al., 2006).

In summary, it can be concluded from our results that active immunization of male pigs against GnRH drastically reduces testicular size and weight as well as the diameter of tubuli seminiferi. Spermatogonia and Sertoli cells were the most numerous cells observed and Leydig cells appeared smaller than in control pigs. Additionally, the length and the diameter of the bulbourethral glands were significantly diminished as a consequence of reduced androgen production in vaccinated pigs. No inflammatory lesions could be found in the hypothalamic and pituitary GnRH-system after vaccination. These findings demonstrate that immunological castration using the anti-GnRH vaccine Improvac® may be regarded as an effective alternative to surgical castration in male pigs without any animal welfare disadvantages.

Acknowledgement

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Observations histomorphologiques et immunohistochimiques des testicules, des glandes bulbo-uréthrales et du cerveau chez des porcelets mâles castrés immunologiquement

Le but du présent travail était l'examen histomorphologique et immunohistochimique des testicules, des glandes bulbo-uréthrales et du cerveau chez des porcelets mâles castrés immunologiquement et leur comparaison avec des mâles non castrés. Quatorze porcelets mâles, âgés de 10 à 16 semaines, ont été traités 2× à un intervalle de 4 à 5 semaines par l'application sous-cutanée d'Improvac®, un vaccin anti-GnRH. Ces porcs ont été abattus entre 1 et 16 semaines après la seconde injection. Chez tous les animaux, le poids des testicules a été mesuré et des fragments de tissus provenant de divers organes ont été prélevés et fixés dans la Formaline ou Boin. Deux animaux supplémentaires ont été traités à 5 reprises et abattus 60 semaines après la dernière injection. L'examen histologique et immunohistologique des testicules et des épididymes montrait des signes d'atrophie clairs. Le poids des testicules des animaux immunisés était abaissé de façon significative. Microscopiquement, le diamètre moyen des tubili seminiferi était fortement réduit et on voyait des spermatogonies ainsi que de rares spermatocytes entre les cellules de Sertoli. Les cellules de Leydig étaient atrophiées. Dans l'épididyme, on ne trouvait aucun ou de très rares spermatozoïdes. Les glandes bulbo-urethrales des porcs castrés immunologiquement étaient plus petites que celles des animaux de contrôle et montraient les signes d'une atrophie. La mise en évidence immunohistologique de LH et de FSH dans l'hypophyse des animaux traités et non traités ne présentait pas de différence quantifiable dans la quantité des deux gonadotrophines et on ne trouvait histologiquement pas de lésions dans l'hypophyse ni dans l'hypothalamus. Cette expérience démontre que le vaccin anti-GnRH Improvac® produit chez les porcs une atrophie importante des testicules et des glandes bulbo-urétrhale. Cet effet semble toutefois être réversible selon l'animal et la durée écoulée depuis la dernière injection de rappel.

Diagnosi istomorfologica e immunoistochimica dei testicoli, delle ghiandole bulbouretrali e del cervello nei suinetti maschi immuno-castrati

Scopo di questo studio è la valutazione istologica e immunoistochimica così come il paragone tra testicoli, ghiandole bulbouretrali e tessuto cerebrale tra verri immunocastrati e non trattati. Quattordici suinetti maschi di età tra le 10 e le 16 settimane sono stati trattati per due volte a distanza di 4 o 5 settimane in modo subcutaneo con Improvac®, un vaccino anti-GnRH. I verri sono stati quindi macellati da 1 a 16 settimane dopo la seconda iniezione. In tutti gli animali sono stati pesati i testicoli e sono stati prelevati campioni di tessuti di diversi organi per l'analisi istologica quindi sono poi stati fissati in formalina o liquido di Bouin. Due animali supplementari sono stati immunizzati cinque volte e solo 60 settimane dopo l'ultima iniezione, macellati. I risultati istologici e immunoistologici dei testicoli e dell'epididimo mostravano chiari segni di atrofia. Il peso dei testicoli degli animali immunizzati era significativamente diminuito. Microscopicamente, il diametro medio dei tubuli seminiferi era molto ridotto e tra le cellule di Sertoli erano visibili spermatogoni e alcuni spermatociti. Le cellule di Leydig erano atrofiche. Nell'epididimo sono stati ritrovati nessuno o pochi spermatozoi. Le ghiandole bulbouretrali di maiali immuno-castrati erano più piccole di quelle degli animali di controllo e mostravano segni di una atrofia. La prova immunoistologica di LH e FSH nell'ipofisi di maiali trattati e non trattati non indica differenze quantificabili nella quantità di entrambe le gonadotropine inoltre l'ipofisi e l'ipotalamo si presentavano, sotto il profilo istologico, esenti da lesioni riconoscibili. Le nostre analisi mostrano che il vaccino anti-GnRH Improvac® provoca nei maiali immunizzati una forte atrofia dei testicoli e delle ghiandole bulbouretrali. Questo effetto risulta però essere reversibile a seconda dell'animale e del periodo temporale dopo l'ultima iniezione Booster.

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