doi: 10.17221/121/2017-VETMED

# Effect of early immunocastration on testicular histology in pigs

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**ABSTRACT**: The objective of this study was to investigate short- and long-term effects of early immunocastration with Improvac® vaccine, administered in two doses, at ages eight and 14 weeks, on testicular histology in pigs slaughtered eight or 15 weeks after the second dose. We hypothesised that the effectiveness of early vaccination could be diminished by late application of the booster dose and/or delayed time of slaughter. Thirty non-castrated male pigs of a commercial hybrid breed were used in this study. Pigs ($n$ = 15) in the control group (NOCA) remained intact throughout the study. Pigs ($n$ = 15) in the experimental group (IMCA) were administered Improvac in two doses: a priming dose at eight weeks and a booster dose at 14 weeks. Subsequently, nine of the IMCA pigs were slaughtered at eight weeks and the remaining six at 15 weeks after the second dose. In NOCA pigs, we observed normal spermatogenesis in the tubuli seminiferi and many prominent interstitial endocrine (Leydig) cells. In $IMCA_8$ pigs, there was a noticeable decrease in the diameter and area of seminiferous tubules and spermatogenesis was absent. Interstitial endocrine cells appeared atrophied with pyknotic nuclei. In $IMCA_{15}$ pigs, we observed a larger diameter of tubuli, thickened germinal epithelium and larger and more numerous interstitial endocrine cells when compared to $IMCA_8$. In conclusion, these results demonstrate that early immunocastration with Improvac disrupts spermatogenesis and reduces the number and size of interstitial endocrine cells. This indicates that vaccination at an age of eight weeks and again at 14 weeks in pigs causes disruption of testicular histology and spermatogenesis at least through the subsequent 15 weeks.

**Keywords**: testis; structure; microscopy; pig; Improvac

In pork, meat taint is a common and unpleasant odour caused by the accumulation of 16-androstenone and skatole in the fat of male pigs. The androstenone steroids (5α-androst-16-en-3-one) are synthesised primarily in interstitial endocrine (Leydig) cells of pig testes and transported to fat tissue (Patterson 1968). Skatole is produced in the large intestine by microbial breakdown of the amino acid tryptophan originating from dietary or endogenous protein (Wilkins 1990). The presence of these compounds significantly affects carcass and meat quality, traits that commonly play a role in rejection by pork consumers.

In Europe and world-wide, surgical castration of male piglets is the most common method for preventing pig meat taint. Besides castration, breeding and genetic selection have been used as alternative solutions to boar taint (Strathe et al. 2014; Drag

Supported by the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1510233) and CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II.; and realised in CEITEC – Central European Institute of Technology with research infrastructure (Project No. CZ.1.05/1.1.00/02.0068) financed by the European Regional Development Fund.

doi: 10.17221/121/2017-VETMED

et al. 2017). However, surgical castration of male piglets is traditionally practiced in the pork industry, for reasons beyond prevention of meat taint. Indeed, a primary goal of this type of castration is the reduction of undesirable sexual and aggressive behaviour in pigs and the calming of the herd.

Castrations have traditionally been performed without the use of anaesthesia or analgesia, and in recent years many consumers have called for pigs to be managed with less stressful, painful and invasive practices. Consequently, the European Union has introduced a Council Directive 2008/120/EC from December 2008, which specifies that surgical castration of pigs shall only be performed with prolonged analgesia and/or anaesthesia. In 2010, the 'European Declaration on alternatives to surgical castration of pigs' was adopted. The Declaration stipulates that, as of 2018, surgical castration of pigs should be phased out altogether. However, some EU member states (including the Czech Republic) have objected to this requirement although countries using analgesia and/or anaesthesia (Norway, Sweden, Switzerland, The Netherlands) have found this method to be practical and effective. Only a few countries seem to be aiming to meet the deadline for phasing out surgical castration (De Briyne et al. 2016).

Immunocastration constitutes an alternative method for the control of pig meat taint. This method involves the vaccination of all male pigs with a gonadotropin-releasing hormone analogue so that antibodies are produced against gonadotropin-releasing hormone. The hypothalamic-pituitary-gonadal axis is thereby blocked and testicular steroid hormone production ceases (Pauly et al. 2010; Fabrega et al. 2011). For effective immunisation, two doses of the vaccine are administered. The first (primer) dose and second (booster) dose are administered at least four weeks apart. Generally, the second dose should be administered not later than four weeks prior to slaughter (Einarsson et al. 2011).

Immunocastration significantly affects the morphological characteristics of the testes. Past observations show that the size and weight of testes are generally reduced after vaccination (Einarsson et al. 2011; Lealiifano et al. 2011). Histological changes in the seminiferous tubules and interstitium of the testes are also objective indicators of successful castration. These changes include a reduced diameter of the seminiferous tubules, a reduced number of interstitial endocrine cells and a reduction of germinal epithelium (Brunius et al. 2011). These immunocastration effects persist for 22–26 weeks after the second/booster vaccination, as manifested in androstenone and skatole levels, the size of reproductive organs (Einarsson et al. 2011; Han et al. 2017) and histomorphological traits observed using light and/or electron microscopy (Kubale et al. 2013; Li et al. 2015; Han et al. 2017).

It is clear from studies to date that earlier vaccination leads to more dramatic and irreversible changes in the testes than vaccination at later ages. Specifically, there is a decline in testosterone levels, a reduction in testicular and bulbourethral size as well as a reduction in spermatogenesis and in the size and number of interstitial endocrine cells. These dramatic effects are causally linked to the decreased levels of testosterone and other hormones produced by the interstitial endocrine cells, which are below those levels required for spermatogenesis (Ge et al. 2008).

However, a decreased efficacy of the Improvac vaccine has also been observed in the case of an early vaccination scheme. While Wicks et al. (2013) used the primary dose of Improvac at eight weeks, their second dose was administered as late as 20 weeks. In this study, testicular function was observed to be restored in some pigs at about 10 weeks after Improvac immunisation.

Therefore, the effectiveness of early vaccination could be diminished by late application of the booster dose and/or delayed time of slaughter. The question arises regarding the optimal time in an early vaccination scheme for the administration of the booster dose of Improvac and the timing of slaughter.

The objective of the present study was to investigate short- and long-term effects of early immunocastration with Improvac administered at eight and 14 weeks of age on testicular structure in pigs slaughtered eight and 15 weeks after the second/booster dose of Improvac. In this study, we investigated the possibilities of following a more flexible vaccination schedule.

## MATERIAL AND METHODS

**Animals and protocol**. Thirty non-castrated male pigs of a commercial hybrid Large white × Landrasse (sow) × Duroc (boar) were used in this

doi: 10.17221/121/2017-VETMED

study. Piglets were weaned at 10.5 kg and kept in identical boxes (five animals) in the accredited experimental barns of the Veterinary Research Institute, Brno, Czech Republic. Pigs were fed twice daily with a standard commercial diet A1–A3 according to the weight category. Animal care conformed to the Institute's good care practices protocol. All experimental procedures were approved by the Central Commission for Animal Welfare of the Czech Republic.

The 30 male piglets were randomly divided into two groups. Pigs ($n$ = 15) in the control group (designated NOCA, not castrated) remained intact throughout the study. Pigs ($n$ = 15) in the experimental group (designated IMCA, immunocastrated) were administered Improvac® vaccine (Pfizer Animal Health, S.A., Belgium) by subcutaneous injection in the neck, immediately behind the ear. Improvac was administered in two doses of 2 ml each. The first (priming) dose was administered at an age of eight weeks and the second (booster) dose at 14 weeks. The timing of the first Improvac vaccination was consistent with the manufacturer's recommendation for the timing of vaccination. Subsequently, a portion of the IMCA pigs ($n$ = 9) were slaughtered eight weeks ($IMCA_8$) and the remainder ($IMCA_{15}$; $n$ = 6) at 15 weeks after the second dose of Improvac. All animals were slaughtered under controlled conditions. Live weight at slaughter was 110.7 ± 9.2 kg for $IMCA_8$, 127.4 ± 6.1 kg for $IMCA_{15}$ and 106.1 ± 8.8 kg for NOCA.

**Tissue sampling**. Testicles (without epididymis) were removed at slaughter. Testes were measured individually and were then weighed in pairs at the slaughterhouse.

The microscopic structure of testes was evaluated for all pigs from IMCA and NOCA experimental groups. Testes were cut in half and on the transverse section and tissue samples were collected from the centre, above the mediastinum (Kubale et al. 2013). Two tissue samples were obtained from each animal for light microscopy.

**Light microscopy**. For light microscopy, all samples were fixed in 5% buffered neutral formalin and embedded in paraffin. Samples were cut using a microtome into sections/slices of a thickness of 8–12 µm and stained with haematoxylin and eosin. Approximately 10 microscopic sections were made from each sample (five sections/slide). They were examined using a Keyence VHX-5000 light microscope (Keyence, Osaka, Japan). At least 10 microscopic fields from each microscopic section were digitised with a camera using different objectives (Keyence VH-Z100R 100–1000 × and VH-Z20R 20–200 ×) and then assessed using Keyence software ver. 1.04 (Keyence).

The testis samples were evaluated for the following parameters: seminiferous duct diameter (µm); seminiferous duct area (µm$^2$); height of the germinal epithelium (µm); and, in interstitial endocrine cells, the diameter (µm), nuclear volume (µm$^3$), nuclear area (µm$^2$) and cytoplasm area (µm$^2$). Inasmuch as the nuclei of interstitial endocrine cells in pigs are spherical, the nuclear volume of interstitial endocrine cells was calculated from the mean nuclear diameter, following Avelar et al. (2010). One to five measurements were made for seminiferous tubule parameters and more than five measurements were made for interstitial endocrine cell parameters in one microscopic field.

**Transmission electron microscopy**. Samples for electron microscopy were obtained from two IMCA and two NOCA pigs.

Ultrathin sections of the samples were fixed in 3% glutaraldehyde in cacodylate buffer, post-fixed in 1% $OsO_4$ solution in cacodylate buffer, dehydrated in 50%, 70%, 90% and 100% acetone and embedded in a mixture of Epon 812 (Serva, Heidelberg, Germany) and Durcupan ACM (Fluka, a subsidiary of Sigma-Aldrich). Sections were stained with 2% uranyl acetate and 2% lead citrate and observed at 80 kV using a Philips EM 208 transmission electron microscope (Philips, The Netherlands).

**Statistical analysis**. Results were evaluated using multifactorial analysis of variance (ANOVA) for determining significant sources of variability. The significance of differences between evaluated parameters was tested by Scheffé's method. $P$-values were considered statistically significant at the levels of $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***). The data were processed using STATISTICA 7.1 software (StatSoft CR Ltd, Prague, Czech Republic).

## RESULTS

Immunocastration significantly affected the testes macroscopically, microscopically and ultramicroscopically.

Macroscopically, the testes of $IMCA_8$ and $IMCA_{15}$ pigs were significantly smaller and lighter than testes from NOCA pigs (Table 1 and Figure 1).

doi: 10.17221/121/2017-VETMED

Table 1. Testes parameters in immunocastrated and non-castrated pigs. Data are presented as mean ± SD measured in immunocastrated (IMCA) and non-castrated (NOCA) pigs

| Group | Testis length (cm) | Testes weight (g) |
|---|---|---|
| $IMCA_8$ | 4.5*** ± 0.5 | 34.6*** ± 8.2 |
| $IMCA_{15}$ | 4.9*** ± 0.3 | 42.8*** ± 11.9 |
| NOCA | 11.2 ± 0.9 | 587.6 ± 130.7 |

The analysis is between NOCA samples and IMCA samples
***$P < 0.001$; Scheffé's method

## Light and transmission electron microscopy

Although there was individual variation between pigs in terms of testicular morphology, the most important factor was length of time between booster vaccination and slaughter.

In testicular tissue of NOCA, spermatogenesis was fully developed, with all stages of spermatogenesis from spermatogonia to spermatozoa evident. The testicular histology in IMCA pigs, by contrast, was clearly negatively affected. Seminiferous tubules did not contain all developmental stages of spermatogenic cells. Significant changes were also noted in the interstitial space and the morphology of interstitial endocrine cells.

## Germinal epithelium

In NOCA pigs, we observed normal spermatogenesis in the tubuli seminiferi. As is evident in Figure 2, spermatogenesis was fully developed with all stages from spermatogonia to spermatozoa evident in TEM (Figure 3). The relatively thick germinal epithelium


Figure 1. Example of the significantly different testes sizes obtained from non-castrated (NOCA) and immunocastrated ($IMCA_8$) pigs

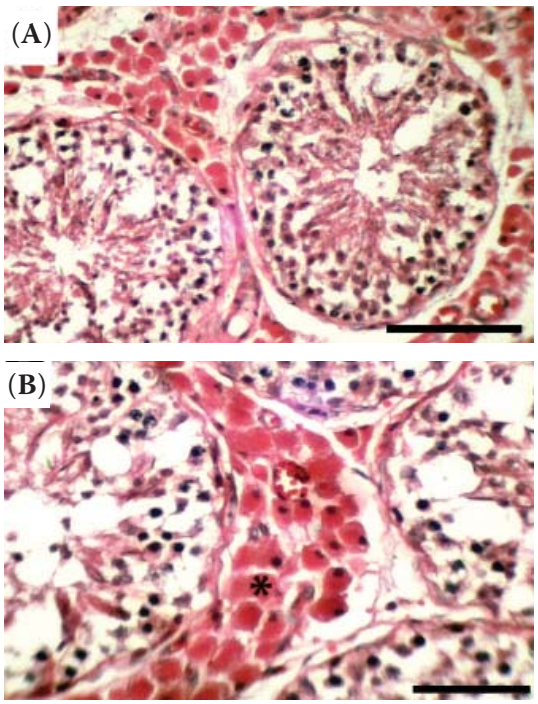


Figure 2. Testicular histology of intact pigs (NOCA) with normal tubuli seminiferi. (**A**) Spermatogenesis products ranging from spermatogonia to spermatozoa are present in tubuli seminiferi (scale bar 100 μm). (**B**) Detail of a large intestitial space with numerous interstitial endocrine cells (*, scale bar 50 μm)

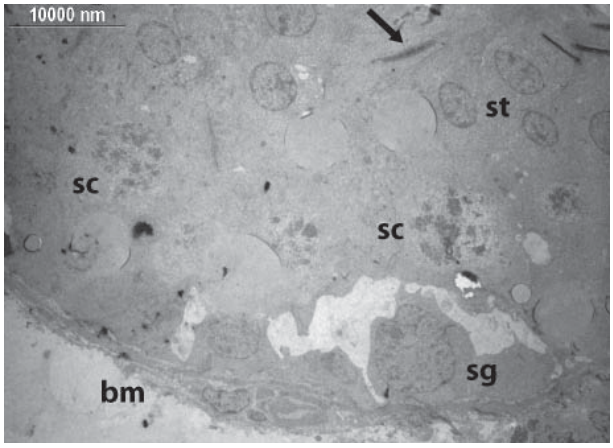


Figure 3. Transmission electron microscopy of non-castrated (NOCA) pigs. Fully developed epithelium with spermatogonia (sg), spermatocytes (sc), spermatids (st) and head of a spermatozoon (arrow). The large space near the base of the germinal epithelium with visible myofibrocytes (bm) is characteristic of NOCA pigs

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Table 2. Testes parameters (seminiferous duct diameter (SDD; μm), seminiferous duct area (SDA; μm$^2$) and germinal epithelium height (GEH; μm)) in immunocastrated and non-castrated pigs. Data are presented as mean ± SD measured in immunocastrated (IMCA) and non-castrated (NOCA) pigs

| Group | Tubuli seminiferi | | |
|---|---|---|---|
| | SDD | SDA | GEH |
| $IMCA_8$ | 91.2*** ± 17.9 | 9803*** ± 93.3 | 33.8*** ± 7.3 |
| $IMCA_{15}$ | 116.3*** ± 27.5 | 13 803*** ± 218.3 | 46.6 ± 16.6 |
| NOCA | 191.4 ± 9.9 | 31 772 ± 76.9 | 64.6 ± 4.2 |

The analysis is between NOCA and IMCA samples. Ten microscopic sections were made from one sample of testis and ten microscopic fields were examined from one microscopic section. One to five measurements were made for each parameter in one microscopic field

***$P < 0.001$; Scheffé's method

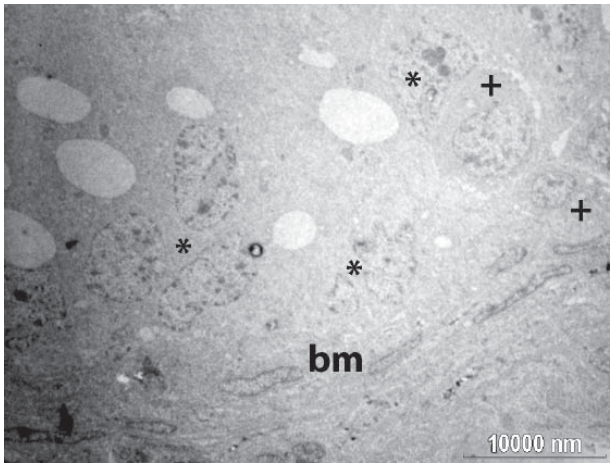


Figure 5. Transmission electron microscopy of immunocastrated pigs. Epithelium of tubuli seminiferi with Sertoli cells with an elongated nucleus (*), vacuoles in the cytoplasm and less numerous spermatogonia (+) can be distinguished. Myofibrocytes (bm) are also present near the base of the germinal epithelium

extended nearly to the centre of the tubuli, and only small lumina remained. The mean diameter of tubuli in NOCA pigs was nearly 200 μm (Table 2).

In $IMCA_8$ pigs, there was a noticeable decrease in the diameter and area of seminiferous tubules (Figure 4). Samples from $IMCA_8$ had approximately half the tubule diameter and one-third the area, compared to NOCA pigs (Table 2); these differences were highly significant ($P < 0.001$). There was no spermatogenesis, as evidenced by the absence of spermatogenic cells at any stage of spermiation in the seminiferous tubules. The *de facto* monolayer epithelium contained only spermatogonia and sustentacular (Sertoli) cells attached to the basal lamina (Figures 4 and 5). In some samples, spermatogonia were sparce. Sustentacular cells had round and elongated nuclei with spotted heterochromatin (Figure 5). In some samples, the tubule lumen was obscured by apical projections of sustentacular cells (Figure 4). The height of the lining epithelium was approximately 8–15 μm.

In $IMCA_{15}$ pigs, we observed only minor changes in the histological structure of tubuli compared to $IMCA_8$ pigs. Notably, these pigs exhibited a larger diameter of the tubuli and thicker germinal epithe-

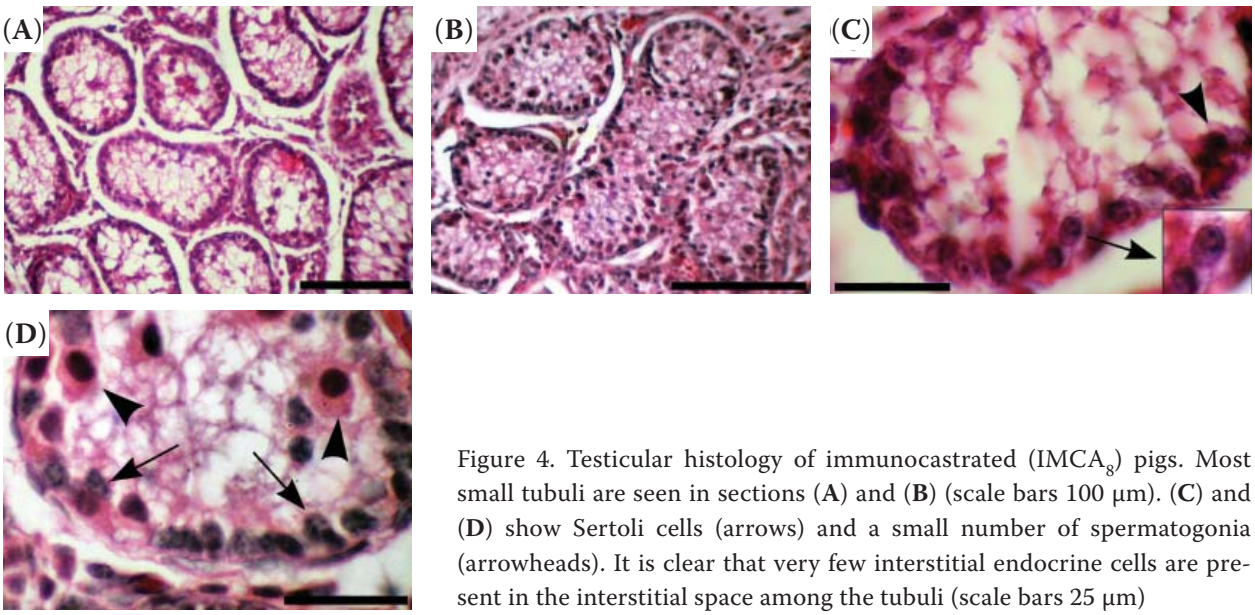


Figure 4. Testicular histology of immunocastrated ($IMCA_8$) pigs. Most small tubuli are seen in sections (**A**) and (**B**) (scale bars 100 μm). (**C**) and (**D**) show Sertoli cells (arrows) and a small number of spermatogonia (arrowheads). It is clear that very few interstitial endocrine cells are present in the interstitial space among the tubuli (scale bars 25 μm)

doi: 10.17221/121/2017-VETMED

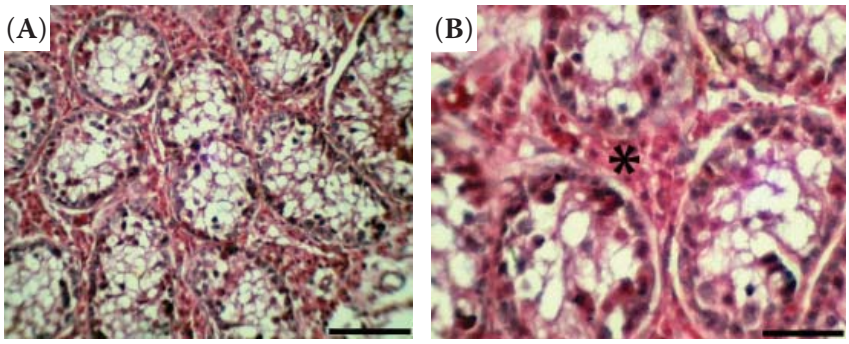


Figure 6. Testicular histology of immunocastrated ($IMCA_{15}$) pigs. (**A**) Tubuli seminiferi with Sertoli cells and some spermatogonia (scale bar 100 μm). (**B**) Interstitial endocrine cells (*) are clearly visible in higher magnification of the interstitium (scale bar 50 μm)

lium (Table 2). Compared to $IMCA_8$ pigs, a "double layer" epithelium was observed containing sustentacular cells and a large number of spermatogonia (Figure 6).

Interestingly, the return of spermatogenesis was noted in one $IMCA_{15}$ pig whose testes were similar in size to those of NOCA pigs: length 9.7 cm and weight 369.2 g. In this case, the diameters of tubuli were 150 μm ± 30 μm and the histological structure of the epithelium was identical to NOCA pigs (Figure 7).

### Interstitial space

In NOCA pigs, the interstitial space contained a large number of prominent interstitial endocrine cells responsible for production of testosterone and performing the main function of triggering the formation of spermatozoa (Figures 2 and 8). Morphometric parameters of these cells are presented in Table 3.

In $IMCA_8$ pigs, immunocastration clearly disrupted the number and morphology of the interstitial endocrine cells (Figures 4 and 9). Interstitial endocrine cells appeared as shrunken and small cells with pyknotic nuclei compared to NOCA pigs (Figures 9 and 10). It was difficult to distinguish interstitial endocrine cells from other cells of the interstitium, e.g., fibrocytes. As noted in Table 3, the nucleocytoplasmic index was noticeably changed and the cell diameter and area of cytoplasm was significantly decreased compared to NOCA pigs ($P < 0.001$).

In $IMCA_{15}$ pigs, we observed higher numbers of interstitial endocrine cells in comparison to $IMCA_8$. Moreover, the interstitial endocrine cells were greater in size and the interstitial space was similar to that seen in NOCA pigs (Figure 6). As shown in Table 3, the area of cytoplasm was nearly twice as large and the nucleocytoplasmic index only half that of NOCA pigs.

## DISCUSSION

In this study, we demonstrated the short- and long-term effects of early immunocastration on testicular structure in comparison to untreated animals. We used Improvac vaccine, administered at eight and 14 weeks, and pigs were slaughtered eight and 15 weeks following the booster dose. Improvac is the first commercial product for the immunocastration of male pigs and is frequently used. The vaccine is the first anti-GnRF product approved for general use in the swine industry. According to

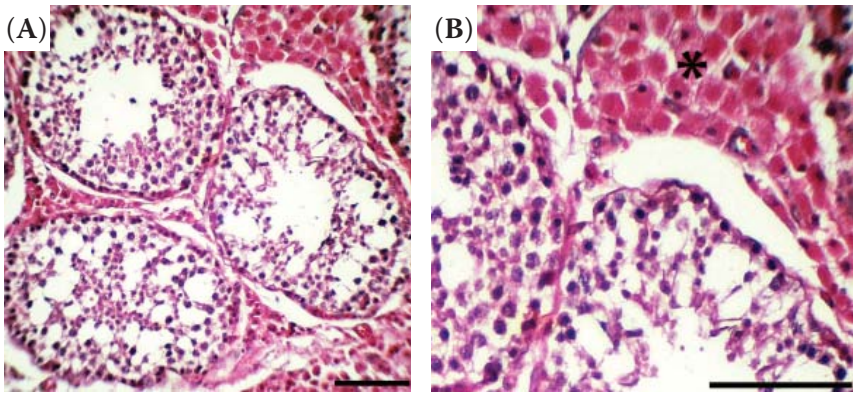


Figure 7. Testicular histology of immunocastrated ($IMCA_{15}$) pigs with recovery of spermatogenesis. (**A**) Note the fully developed epithelium in the tubuli (scale bar 100 μm). (**B**) Moreover, large numbers of interstitial endocrine cells (*) are evident within the interstitium (scale bar 50 μm)

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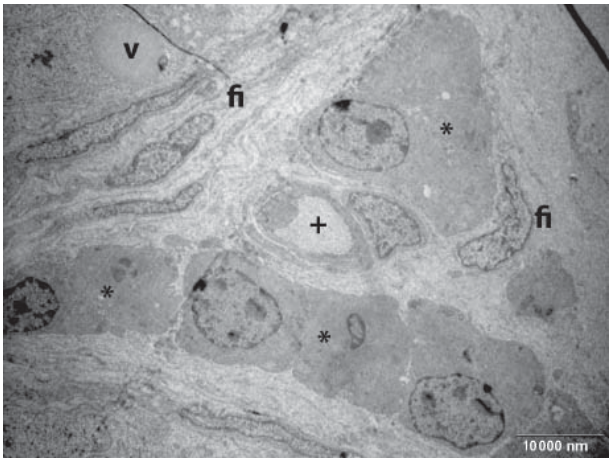


Figure 8. Transmission electron microscopy of non-castrated pigs. The interstitial space with prominent interstitial endocrine cells (*), myofibrocytes (fi) and blood vessels (+). A vacuole is present in the Sertoli cell in the tubuli seminiferi (v)

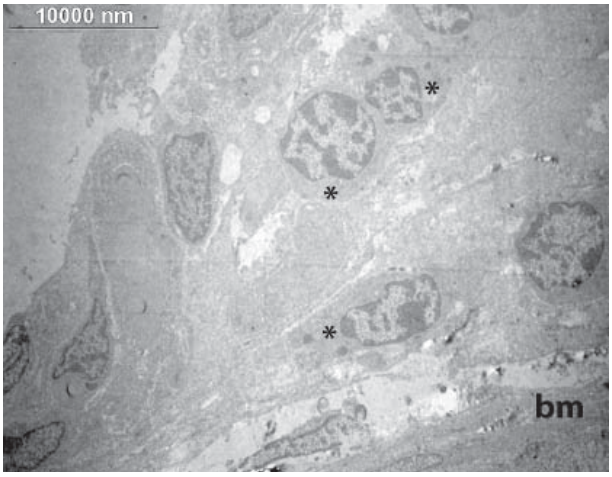


Figure 9. Transmission electron microscopy of immunocastrated pigs. Interstitial endocrine cells (*) with a small amount of cytoplasm and myofibrocytes (bm) are evident in the interstitial space

the manufacturer's instructions, the vaccine's primary dose can be administered no earlier than at eight weeks. In fact, researchers have used various vaccination schedules, with the primer dose being administered at 11–16 weeks and the booster dose at 18–21 weeks (Einarsson et al. 2009; Batorek et al. 2012; Kubale et al. 2013; Li et al. 2015; Shi et al. 2016; Needham et al. 2017). In these cases, the effect of vaccination may persist for as long as 22 weeks (Zamaratskaia et al. 2008; Einarsson et al. 2009).

In addition to the standard vaccination protocol, some investigators have used early (pre-pubertal or early pubertal) vaccination with Improvac (Brunius et al. 2011; Andersson et al. 2012; Lugar et al. 2017). This early vaccination can be used as an alternative to the recommended schedule in order to minimise problematic aggressive behaviour of the males, while having no effect on profitability. Einarsson et al. (2011) administered Improvac at 10 and 14 weeks and observed a long-lasting negative effect on fertility, with none of the animals recovering testicular function, at least up to 25 weeks.

The first sign of immunocastration was a reduction in testes size. It has been reported that immunocastration decreases the size of reproductive organs (Batorek et al. 2012) and we observed very small testes in $IMCA_8$ and $IMCA_{15}$ pigs compared to NOCA pigs. Brunius et al. (2011) reported that a more pronounced reduction in reproductive organ size occurred in pigs vaccinated earlier. Einarsson et al. (2011) stated that the reduction in testis size was most likely the combined result of arrested testicular development at an earlier age and a prolonged period without spermatogenesis. Brunius et al. (2011) and Einarsson et al. (2011) used early

Table 3. Testes parameters in immunocastrated (IMCA) and non-castrated (NOCA) pigs. Data are presented as mean ± SD measured in IMCA and NOCA pigs

| Group | Interstitial endocrine cells | | | | |
|---|---|---|---|---|---|
| | diameter (μm) | nuclear volume ($\mu m^3$) | nuclear area ($\mu m^2$) | cytoplasm area ($\mu m^2$) | nucleocytoplasmic index (nuclear area/cytoplasm area) |
| $IMCA_8$ | 7.1*** ± 0.6 | 27.6 ± 1.5 | 11.1 ± 0.3 | 26.4*** ± 5.4 | 0.42 |
| $IMCA_{15}$ | 8.2 ± 2.2 | 24.4 ± 6.3 | 10.6 ± 2.7 | 42.4* ± 13.4 | 0.25 |
| NOCA | 11.3 ± 0.9 | 32.5 ± 1.6 | 12.3 ± 0.6 | 88.2 ± 16.3 | 0.14 |

The analysis is between NOCA samples and IMCA samples. Ten microscopic sections were made from one sample of testis and ten microscopic fields were examined from one microscopic section. At least five measurements were made for each parameter in one microscopic field

***$P < 0.001$; *$P < 0.05$; Scheffé's method

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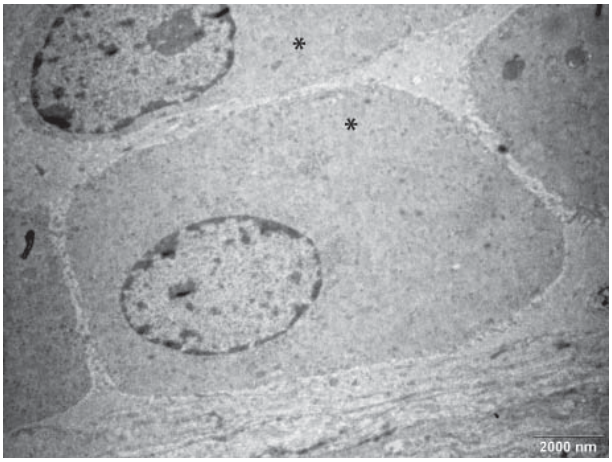


Figure 10. Transmission electron microscopy of non-castrated pigs pigs. Detailed view of interstitial endocrine cells (*) in the interstitial space of testis. A distinct nucleus and a large amount of cytoplasm are apparent

Improvac immunocastration, at 10 and 14 weeks, and the paired testes weights reported were 74 g and 109 g. In our study, we used early immunocastration at eight and 14 weeks and the paired testes weight was approximately 35 g. It is evident that early vaccination dramatically reduces testicular maturation. Improvac was administered at eight weeks, when testicles start to undergo significant differentiation and growth (Avelar et al. 2010). When the second dose of Improvac is administered as late as 20 weeks, paired testes weight can exceed 250 g (Wicks et al. 2013). Interestingly, Batorek et al. (2012) and Han et al. (2017) observed testes weights of about 300 g and 147 g, respectively, for a standard vaccination scheme of 10–12 weeks and booster dose at 18 weeks.

In addition to macroscopically detectable changes, Improvac markedly affected the histology of testes in $IMCA_8$ and $IMCA_{15}$ pigs. Histological changes are related to inhibition of hypothalamic synthesis and secretion of gonadotropin-releasing hormone, which normally stimulates adenohypophyseal production and release of follicle-stimulating hormone and luteinising hormone. Luteinising hormone is responsible for initiating testosterone production by the interstitial endocrine cells. Follicle-stimulating hormone and testosterone stimulation of the sustentacular (Sertoli) cells initiates spermatogenesis and spermiation (Basu et al. 2017). In the absence of these hormones, normal stimulation of the testes is compromised; gonadal regression and infertility follow (Miller et al. 1997).

In accordance with earlier studies, Improvac vaccination significantly disrupted spermatogenesis and decreased the number and size of interstitial endocrine cells (Hilbe et al. 2006; Einarsson et al. 2009, Einarsson et al. 2011). We also observed these changes in our study, but our study differed in relation to the length of time from booster dose administration to slaughter. The most noticeable changes were observed in $IMCA_8$ pigs as opposed to in $IMCA_{15}$ animals. In this case, we observed smaller seminiferous duct perimeter and height of the germinal epithelium along with inhibited spermatogenesis. In $IMCA_8$ samples, only a few spermatogonia were present alongside sustentacular cells, suggesting that spermatogenesis was disrupted reversibly. Others have observed tubuli with only sustentacular cells, indicating an irreversibility of spermatogenesis (Einarsson et al. 2011). In our $IMCA_{15}$ samples, we observed large numbers of spermatogonia, suggesting that these cells were mitotically active and could potentially act to restore spermatogenesis. Overall, the histology of germinal epithelium in IMCA pigs was similar to that seen prior to puberty. The structure of sustentacular cells also suggests this. In IMCA samples, sustencacular cells had round and elongated nuclei, a pattern which is characteristic for pre-pubertal ages (Avelar et al. 2010).

Alterations in the morphology and functionality of the tubuli seminiferi coincide with changes observed in interstitial endocrine cells (Kubale et al. 2013). In our study we observed significant changes in interstitial endocrine cells in immunised pigs. Similar to changes seen in sustentacular cells and spermatogonia, the extent of changes seems proportional to the time remaining until slaughter. In $IMCA_8$ pigs, immunocastration caused a reduction in the number and size of interstitial endocrine cells. In some cases, these cells were nearly indistinguishable from other cells of the interstitium. Interstitial endocrine cells were shrunken and had both a small nucleus and limited cytoplasmic area. Similar results have been reported by other studies (Einarsson et al. 2011; Kubale et al. 2013; Li et al. 2015). These changes in the morphology of interstitial endocrine cells were consistent with a loss of function and the decrease in testosterone and androstenone (Kubale et al. 2013).

In $IMCA_{15}$ samples of our study, the size of interstitial endocrine cells and their numbers were greater; the histology of the interstitial space was

doi: 10.17221/121/2017-VETMED

similar to that of NOCA pigs. These changes – apparent 15 weeks after the second dose of Improvac – strongly suggest progression toward a return of spermatogenesis. Moreover, in a single $IMCA_{15}$ pig there was a return to spermatogenesis. The histology was nearly the same as in NOCA pigs. This is not surprising inasmuch as a return to spermatogenesis after Improvac vaccination has also been observed in other studies. Kubale et al. (2013) reported that the testicles from animals administered vaccine four months prior to slaughter showed recovery of spermatogenic function. Similarly, Wicks et al. (2013) reported that testicular function in boars treated with Improvac began to recover 10 weeks after immunisation. It should be noted that these laboratories used different vaccination protocols. Kubale et al. (2013) did not use an early vaccination scheme, and the first dose was administered at 12 weeks. Wicks et al. (2013) used an early vaccination protocol, but the booster dose of Improvac was administered only 12 weeks after the first dose. In contrast, Einarsson et al. (2011) reported a long-lasting disruption of spermatogenesis in pigs vaccinated at as early as 10 and 14 weeks. Moreover, these investigators noted a persisting effect of immunocastration up to 25 weeks. Again, it is evident that the histological changes in testes are more prominent in pigs vaccinated early. This is consistent with the results of our study.

In conclusion, our hypothesis that the effectiveness of early vaccination could be diminished by late application of the booster dose and/or delayed time of slaughter was not confirmed. The results reported in this study demonstrate that early immunocastration using Improvac leads to a reduction in the size of testes, disruption of spermatogenesis as well as a decrease in the number and size of interstitial endocrine cells. This indicates that vaccination at ages of eight and 14 weeks in pigs causes disruption of testicular structure and function through the subsequent 15 weeks. This time-related effect allows the use of a more flexible vaccination schedule.

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doi: 10.17221/121/2017-VETMED

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Received: September 18, 2017
Accepted after corrections: December 12, 2017