MORPHOLOGICAL STRUCTURES IN GERMINAL EPITHELIUM AFTER IMPROVAC APPLICATION IN PIG

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Abstract: Immunocastration of pigs is an alternative way to prevent the presence of boar taint, especially skatole which cause negative deviation of meat quality. The vaccine Improvac actively immunizes against gonadotropin – releasing hormone (GnRH). It triggers a cascade of hypotalamic – pituitary – gonadal axis actions during sexual maturation and contributes to the development of spermatogenesis. This inhibition leads to significant changes in testicular tissue, both structural and functional. Testes undergo changes effecting reproduction in addition to other indicators, such as boar taint. For comprehensive understanding of this topic is needed specifically specify changes in testis tissue and possible impacts in other economically interesting tissues. In the experiment 10 boars were treated with the vaccine Improvac with two subcutaneous applications. The testes were processed by standard histological techniques. Testes tissue have showed significant morphological changes in seminiferous tubules and interstitial space. Null or decreased spermatogenesis were performed in germinal tissue and atrophy of Leydig cells leaded to inhibition of steroidogenesis.

Key Words: immunocastration, testicles, histological structure, light micropscopy

INTRODUCTION

Today castration of pigs is a frequent topic professional public. There are three possible ways how to prevent boar taint in porcine meat: untimely slaughter, surgical castration or immunocastration by immunization against gonadotrophin – releasing hormone (GnRH). The most discussed has been surgical castration, which presents problem in term of welfare (Brunius et al. 2011). On the basis of physiological and etological parameters is known that surgical castration presents a painful treatment, even is done in young animals (Kubale et al. 2013).

Current European legislation (Council Directive 2008/120/EC laying down minimum requirements for the protection of pigs) authorize the implementation of castration only qualified persons, i.e. veterinarians or trained persons (European union 2008). Surgical castration is performed in young animals – without anesthesia and can be performed within 7 days after birth, after this period must be used during surgical castration anesthesia and subsequent pain relief. Opinion of the Scientific Panel on Animal Health and Welfare Animal European Food Safety Authority (EFSA) in 2004, however, indicates that surgery castration is painful at any age. That's why many European countries have decided to surgery castration retreat and replace it with other, non-painful methods. One of the first steps is the introduction of analgesia or anesthesia to alleviate pain, which has been used since 1 January 2012. The next step, to which European countries committed themselves to the phasing out of surgical castration. This way prevention of boar taint should stop using until 1 January 2018 respectively (EFSA 2004).

For this reason, there are alternative ways to approach prevention of boar taint (Batorek et al. 2012). One of these methods is immunocastration with vaccine Improvac, which is beeing examined in this work. Introduction of new methods in practice requires comprehensive knowledge of the physiological status of the animal. The effect of the vaccine is directed to eliminate boar taint, but primarily affects the histological structure of testes (Brunius 2011). Therefore, the aim of this study was histological analysis of the germinal epithelium of the pig testes treated with the vaccine Improvac.
MATERIAL AND METHODS

Animals

The total of 10 male pigs was included in the study. Rearing animals were carried out in accredited stables of Veterinary Research Institute in Brno (VRI, Czech Republic).

Experimental design

Experimental boars were administered with two consecutive Improvac vaccine in the range of 43 to 46 days, wherein the second vaccine was administered 2–4 months before slaughter. Age at slaughter ranged from 22–29 weeks. Live weight before slaughter ranged from 78.8 kg to 100.2 kg.

Examination post mortem

The experimental material was collected by trained staff from authorized and registered slaughterhouse at VRI. Samples were collected from the testicle tissue among the mediastinum testis and tunica albuginea in the range of 10 mm x 10 mm x 10 mm to 10% formalin. The samples were processing according to standard histological techniques as are processing, embedding, sectioning and hematoxilin-eosin staining protocol (Bancfort et al. 2008). Sections were mounted in permanent preparations. The histological slides were evaluated using the light microscope (Olympus BH–2, Japan) a morphometry was determined after digitizing microscope slides (camera Canon EOS 1100D, Japan) using software (Quick Photo Micro 3.0, PROMICRA, Czech Republic).

The total of 20 histologic slides were analysed, each slide had 5 sections and evaluated in duplicate magnification 100x and 400x. Following parameters were evaluated : diameter of coiled seminiferous canal, height germinal epithelium, number and nucleocytoplasmatic index of Leydig cells, the presence of mature spermatids in the lumen of the seminiferous coiled duct (Kubale et al. 2013).

RESULTS AND DISCUSSION

The testicular histology in vaccinated male pigs was clearly affected. The data obtained from histological sections showed the distribution of the seminiferous tubules in the testes tissue in cross section, with a density 3–5 tubules per 100 µm². Individual variation was presented in testicular morphology among pigs. Important factor is length of period since vaccination to slaughter. Seminiferous tubules do not contain all the developmental stages of spermatogenic cells. Significant changes are noticeable in interstitial space and on morphology of Leydig cells.

Germinal epithelium

Conclusive decrease in area of seminiferous tubules was observed on diameter 50 µm ± 10 µm in the samples taken from animals administrated with immunovaccine 2 months before slaughter. Spermatogenesis was inhibited, as evidenced by the absence of higher stage of development of spermatogenic cells into the lumen of the seminiferous tubules. On preparations only spermatogonia and non distinctly Sertoli cells to the basement membrane were visible, see figure 1B. Lumen was not formed inthe center of seminiferous duct. Apical poles of Sertoli cells fill the entire ducts. From this description it is obvious that the seminiferous coiled tubules cannot contain spermatids, which undergo metamorphosis in mature sperm. For this reason it is difficult to measure height of germinal epithelium, even if it is not mature. On the other hand testicles tissue from animals administrated with vaccine 4 months before slaughter have showed recovery of spermatogenic function, the same conclusion reached Kubale et al. (2013). Their diameters were 150 µm ± 30 µm and all levels of developmental stadium were presented, see figure 2D. On the other hand in the study of Einarsson et al. (2011) was indicated long-lasting disrupting effect on histological status in male pigs vaccinated as early as at 10 and 14 weeks of age.
Figure 1 Histological structure of testes tissue after immunocastration with Improvac vaccine

![Image of histological structure](image)

Legend: Light microscopy photographs of testicular tissue of immunocastrated pigs. Figure A shows seminiferous coiled tubules of male pig administrated with second vaccine of improvac 70 days before slaughter in magnification 100x. No lumen of seminiferous tubuli and reduced interstitial space, number and size of Leydig cells were presented. B Spermatogenesis was disrupted here, only spermatogonias as a first developmental stadium and Sertoli cells were observable in magnification 400x.

*Structure of histological sections are slightly disrupted by processing of technical process.

Interstitial space

In non-castrated pigs interstitial space contains Leydig cells, which are responsible for the production of testosterone and perform the main function of the trigger formation of male sex cells. Improvac vaccine clearly disrupted the number and morphology of the interstitial Leydig cells. Hilbe et al. (2006) have evaluated Leydig cells as well as atrophic. The size of the interstitial space in immunocastrated males has been significantly reduced. The Leydig cells lost their pycnotic-like nuclei and they were difficult to distinguish from the other interstitial structures. Nucleocytoplasmatic index of Leydig cells is noticeably altered. The volume of the cytoplasm decreased, see figure 1A, on the other hand tissue with renewed spermatogenesis has showed specific polygonal shape of Leydig cells which represent their activity, see figure 2C.
Figure 2 Histological structure of testes tissue after immunocastration with Improvac vaccine

Legend: All stadiums of spermatogenesis were showed at figure "C" in magnification 100x and the lumen * of seminiferous tubuli is presented here. The tissue sample was taken from pig immunocastrated with the second vaccine 111 days before slaughter. The arrow marks Leydig cells with their specific polygonal shape. "D" Spermatogonia cells adjacent to the basement membrane, primary and secondary spermatocytes and spermatids were distinguishable in magnification 400x.

* Structure of histological sections are slightly disrupted by processing of technical process.

CONCLUSION

This histological study has showed clear signs of atrophy in the immunized animals. At the morphological level this alternative method of castration has proved the effectiveness and applicable in practice. Based on examination the significant effects of the vaccine is believed to be temporary as appeared spontaneous spermatogenesis in testicular tissue of pigs vaccinated with 4 month interval. The temporal boundaries of recovery process are not known. The question remains how long after immunocastration production of skatole is inhibited even if the spermatogenesis is resumed?

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