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# Immunocastration as Alternative to Surgical Castration in Pigs

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Additional information is available at the end of the chapter

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## Abstract

Surgical castration of piglets is a routine practice in pig production used to prevent the incidence of boar taint of pig meat, which may develop in entire male pigs as they reach puberty. This practice is being presently questioned in the European Union, and there is a strong initiative to end it. The initiative is presently voluntary; however, key stakeholders of European pig production sector have signed a declaration, and the actions undertaken by them already affect the business. Before such new concepts in pig production can be implemented, alternative solutions are needed, one of them being immunocastration. The present chapter will thus focus on the presentation of immunocastration as one of the promising alternatives to surgical castration. Theoretical and practical aspects of immunocastration in pig production will be described, and the advantages and disadvantages of this alternative will be summarised. Physiological principles of immunocastration and impacts on metabolism, growth performance, body composition and meat quality will be described and aspects of public acceptability reviewed.

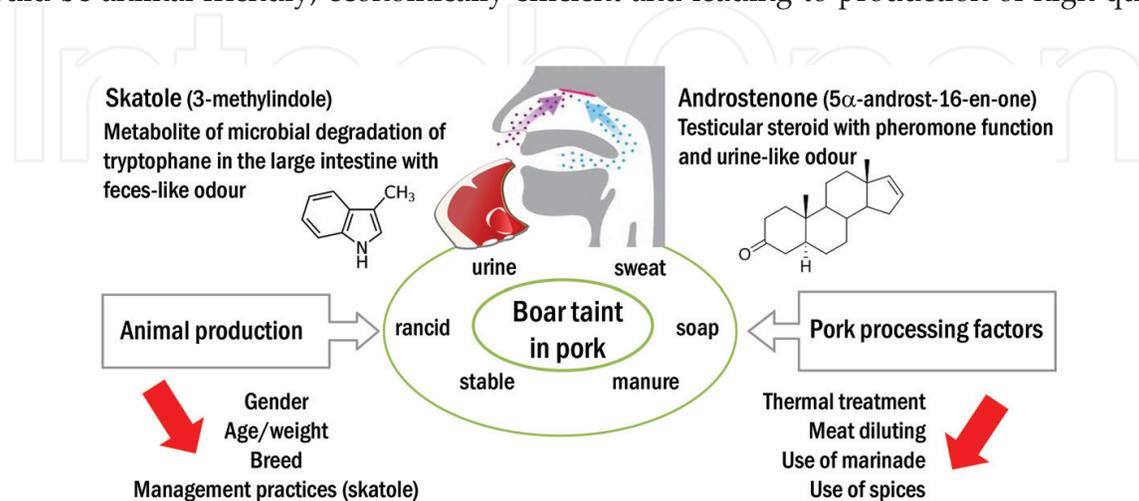
**Keywords:** immunocastration, productivity, welfare, meat quality, public acceptance, pigs

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## 1. Introduction

Castration of male piglets is a traditional practice in pig production used worldwide with the main goal to prevent boar taint of pig meat—an unpleasant odour refused by the majority of consumers [1]. Odour is an important sensory attribute that determines whether consumers will be satisfied with a meat product. In pork, odour can be adversely affected by accumulation of high levels of androstenone and/or skatole, the so-called boar taint [2, 3]. Androstenone

is a testicular steroid (with no anabolic effects) and is described as having urine or sweat-like odour. It is produced by testicular Leydig cells of sexually mature males. Due to its lipophilic character, it accumulates in adipose tissue in much higher concentrations than other steroid hormones [4]. Androstenone is also secreted through saliva and serves as a pheromone to promote sexual behaviour in sows. On the other hand, skatole is produced in the intestine; its odour is related mostly to manure or, to a lesser extent, to naphthalene. Skatole has no known physiological function; it is toxic for most animals, but pigs are relatively resistant to it. It is a product of bacterial degradation of the amino acid tryptophan in the large intestine and is partly excreted through faeces, while the rest is absorbed in the blood and metabolised in the liver. Its hepatic metabolism is inhibited by steroid hormones (including androstenone). As a result, the increased concentrations of androstenone are responsible for higher levels of skatole [5]. Likewise skatole, due to its lipophilic nature, accumulates in the adipose tissue. The fat levels above which the consumers can detect the off-odour were determined to be in the range from 0.5 to 1.0 ppm for androstenone and in the range 0.2–0.25 ppm for skatole [6]. The major aspect determining the level of boar taint in pork is the balance between the biosynthesis and catabolism of androstenone and skatole. This balance is affected by various intrinsic and extrinsic factors (**Figure 1**) influenced mainly by pig genotype and nutrition (for review, see Refs. [5, 7]). Until recently, a traditional way to regulate boar taint was to modify gender by surgical castration of male pigs. Surgical castration prevents the formation of both androstenone and skatole; however, it is associated with productivity drawback, as it ceases the synthesis of testicular steroids including testosterone and oestrogens and therefore negatively affects lean tissue growth and feed efficiency. According to the legislation of the European Union (EU), surgical castration can be performed without the use of analgesia/anaesthesia within the first week after the birth of piglets [8]. Due to the pain induced during the procedure, there is a growing public criticism of this practice from pig welfare point of view [9, 10]. Thus, both economic and ethical concerns make it relevant to reconsider the need for surgical castration. As a consequence, a voluntary initiative has been launched by key stakeholders to stop castrating male piglets in the EU until 2018 [11]. However, to be able to stop castration, alternative methods are required to minimise the risk of boar taint. Ideally, these methods should be animal friendly, economically efficient and leading to production of high-quality



**Figure 1.** Boar taint: descriptors, responsible substances and influential factors.

and nutritious products. Among existing alternatives to surgical castration (**Table 1**), the so-called immunocastration, an active immunisation against gonadotropin-releasing hormone

Alternative		Advantage	Disadvantage
Castration	Surgical castration with anaesthesia and/or analgesia	Reduced pain during surgical castration	Increased costs, need for authorisation (drugs) and specially trained personnel
	Immunocastration	No castration pain and wounds Applicable for males and females Economic advantage of better performance Applicable for production systems with prolonged fattening	Need for authorisation (drugs) Need for safety measures for operators (self-injection) Questionable acceptability for consumers (and consequently chain actors)
Raising entire male pigs	Slaughter at younger age/ lower weight (before puberty)	No conflict with animal welfare Reduced risk of high androstenone and skatole levels Economic advantage of better performance	No guarantee of total elimination of boar taint Lower technological meat quality Questionable economic efficiency
	Dietary manipulations	No conflict with animal welfare Reduced risk of high skatole levels Economic advantage of better performance	No guarantee of total elimination of boar taint Lower technological meat quality High costs of specific ingredients Not a solution for production systems with prolonged fattening
	Selection against boar taint	No conflict with animal welfare Reduced risk of high androstenone and skatole levels Economic advantage of better performance	No guarantee of total elimination of boar taint Lower technological meat quality Not a solution for production systems with prolonged fattening Reduced levels of anabolic hormones and, therefore, negative effects on growth performance of entire male pigs and onset of puberty in male and female pigs
Sex sorting	Sperm sexing	Production of female-only herds	High costs, low sperm output Technique for gender selection is not commercially available

**Table 1.** Cost-benefit analysis of available alternatives as compared to standard surgical castration of entire male piglets.

(GnRH; also referred to as gonadoliberin), is considered as an appropriate and one of the most attractive alternatives. Immunocastration hinders sex steroid synthesis, including androstene production, along with a reduction of the size of reproductive organs, sperm number and aggressive behaviour [12–17]. Skatole levels are also reduced by immunocastration [13, 17–19]. The principle of immunocastration is based on the immunological blocking of the signal from GnRH, thus decreasing the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) and testicular steroids.

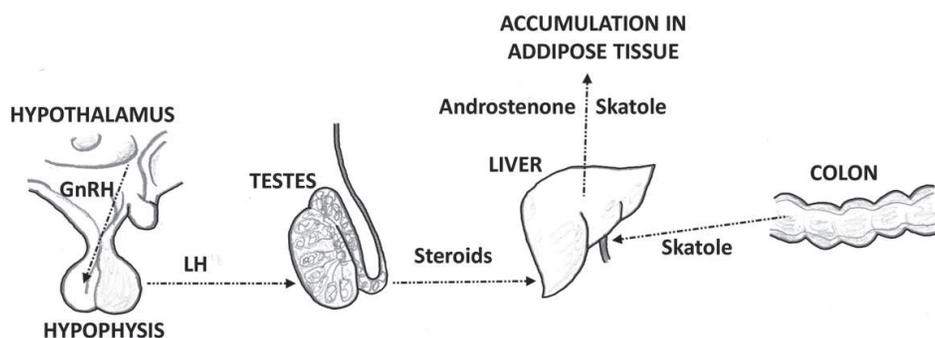
## 2. Reproductive physiology of boar

Puberty can be defined as series of physiological changes leading to full sexual maturity and capability of reproduction. It is accompanied by changes in testes structure and increased secretion of androgens and oestrogens. Puberty is heralded by an increase in the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland. These processes are controlled by the extent and frequency of GnRH pulses, along with the feedback from androgens and estrogens. LH and FSH are responsible for the regulation of testicular function. The binding of LH to the receptors on the surface of the Leydig cells results in the induction of steroidogenic enzymes and increased levels of testicular steroids including androstenone. FSH affects the functioning of testicular Sertoli cells and is critical for the initiation of spermatogenesis. LH secretion is also controlled by some other hormones such as dopamine and prolactin and most crucially by negative feedback from sex steroids. It has also been shown that Leydig and Sertoli cells have receptors for growth factors including IGF-I [20]. In boars, growth hormone also stimulates functional maturation of Sertoli cells although without an effect on their number [21]. Thyroid hormones are also critically important for normal testicular development (of Sertoli cells and testes as a whole) [22]. Age-related variations of androstenone and testicular hormones are due to the common regulatory system controlling the biosynthesis of all testicular steroids. The synthesis of androstenone is low in young pigs (the transient increase in androstenone levels also occurs at the age of approximately 2–4 weeks due to Leydig cell activity at that time) but gradually increases simultaneously with other testicular steroids at puberty onset [23]. Therefore, puberty is a central stage of development regulating androstenone levels in entire male pigs by the maintenance of adult Leydig cell morphology and the stimulation of neuroendocrine system leading to increased biosynthesis of testicular steroids (mature boars show an increase in average Leydig cell size and therefore an increase in steroidogenic capacity per Leydig cell). In sexually mature boars, androstenone levels depend on the individual ability to produce this steroid.

In entire male pigs, androstenone is produced by the Leydig cells of the testes in parallel with anabolic testicular hormones [24]. Androstenone is synthesised from the precursors, pregnenolone and progesterone, through the formation of androstadienone by the sequential action of a number of enzymes, particularly cytochrome P450C17 and cytochrome b5 [25, 26]. Androstenone is metabolised in the liver with the production of alpha-androstenol and to a greater extent beta-androstenol [27, 28]. Part of androstenone is transported to the submaxillary salivary gland, where it is bind to a specific binding protein pheromaxein and released in

the saliva, which among other 16-androstene steroids act as a pheromone to promote sexual behaviour in female pigs.

Hormonal regulation of boar taint is illustrated in **Figure 2**, which shows how androstenone biosynthesis is controlled through the activation of the hypothalamic-pituitary-gonadal axis. The level of skatole, the other boar taint compound, is also related to sexual maturation. Its accumulation in the adipose tissue is due to the inhibition of skatole metabolism in the liver by increased levels of testicular steroids, mainly androstenone [29] and oestrogens [30, 31], and in part also due to the effect of steroid hormones and growth factors on the epithelial proliferation and apoptosis in the intestine, the site of skatole formation [5].

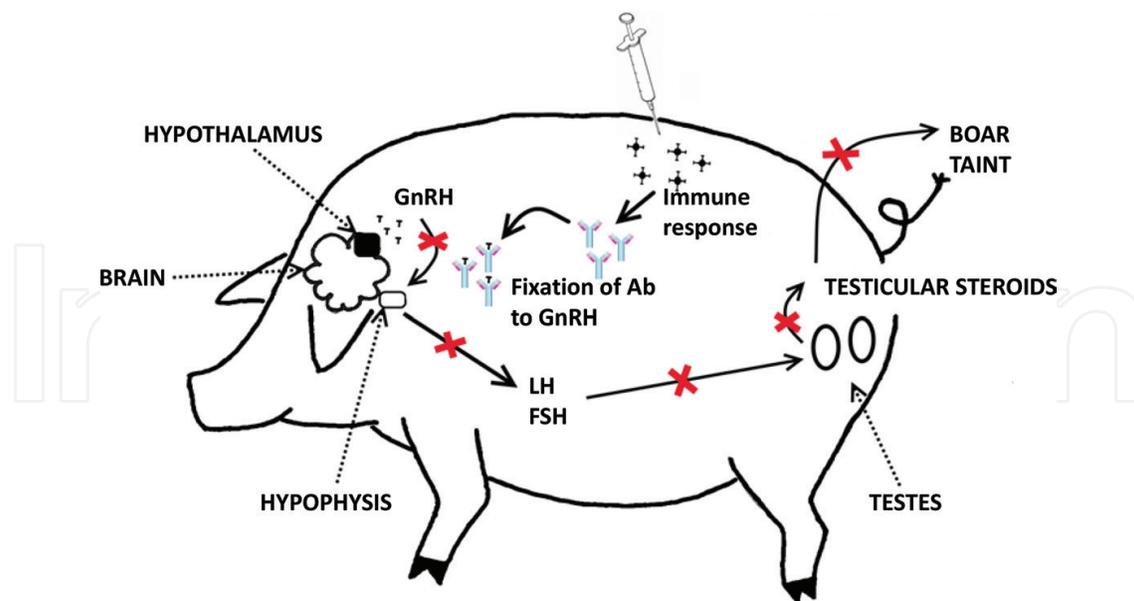


**Figure 2.** Relationships between the hypothalamic-pituitary-gonadal axis, androstenone production in testes and skatole formation from tryptophan in the intestine and their interrelated metabolism in the liver. In boar, the production of testicular steroids, including androstenone, inhibits hepatic clearance of skatole. Androstenone and skatole are accumulated in the adipose tissue due to their lipophilic nature.

### 3. Principles and effects of immunocastration

Immunocastration involves the vaccination of animals against hormones that control the reproductive function (**Figure 3**). Progress has lately been made to develop a vaccine for the immunisation against gonadotrophin-releasing hormone (GnRH). Commercially available vaccine (named Improvac in Europe, Improvest in the USA) against boar taint was developed in Australia and is now produced by Zoetis (formerly Pfizer Ltd., formerly CSL Limited, Parkville, Victoria, Australia). This vaccine was approved for use in pigs in many countries (including the EU from 2009), but its practical application is still limited.

Immunocastration uses the natural immune system of the animal to achieve the effects of castration. The vaccine contains physiologically inactive analogue of GnRH covalently conjugated to an immunogenic carrier protein. The analogue has no hormonal activity but contains the necessary epitopes to stimulate an effective anti-GnRH antibody response and blocks the stimulation of the hypothalamic-pituitary-gonadal axis. Consequently, the formation of gonadal steroid hormones is hindered and thereby the regression of reproductive organs and some induced metabolic changes, which ultimately leads to changes in behaviour (reduced aggression, increased appetite and feed intake) and growth performance [32].



**Figure 3.** Physiological response to immunocastration in male pigs. The vaccine consists of the antigen (GnRH analogue that binds to carrier protein), which triggers the immune system to produce antibodies that neutralise endogenous GnRH. Consequently, there is no stimulus for the hypophysis to release LH and FSH hormones, which in turn fails to signal the testes to produce testosterone and androstenone and thus prevents boar taint development.

### 3.1. Vaccination scheme

To achieve the effective immunisation, at least two applications of the vaccine with a minimum interval of 4 weeks are needed. Subcutaneous injections are given at the base of the ear with a special vaccinator designed by the vaccine producer to prevent accidental self-injection. The first dose primes the pig's immune system and can be given at any time after 8–9 weeks of age, and the second dose should be given (if we refer to standard pig production system where pigs are slaughtered at 6 months of age) no later than 4–5 weeks prior to slaughter. As the first injection has no apparent impact on steroid hormones, this schedule enables to use full growth potential of the entire male pigs until the second injection. After the immunisation, immunocastrated pigs rapidly change their metabolism to castrate-like, with increased feed consumption and fat deposition. The longer is the time elapsed from the second vaccination to slaughter, the higher is the difference between immunocastrates and entire males and/or the similarity to surgical castrates [33, 34]. In the case of older animals, a three-dose vaccination regimen might be required [35, 36] to ensure inactivation of endogenous GnRH and elimination of boar taint. Also, if nonrespondent pigs are detected (shown as larger testicle size or prolonged sexual behaviour), an additional dose might be applied [37].

A number of studies have been conducted using alternative vaccination schemes. A study conducted by Brunius et al. [38] investigated the efficacy of early vaccination with Improvac applied to entire male pigs at 10 and 14 weeks of age (pre- or early pubertal). It was shown that the levels of androstenone and skatole in pigs vaccinated at weeks 10 and 14 did not differ from the pigs vaccinated according to manufacturer's instructions. It has also been shown that already 2 weeks following the second vaccination, the levels of androstenone and skatole were below sensory threshold [33, 39]. The effect of immunocastration can last up to 22 weeks following the second injection [19].

### 3.2. Effect of immunocastration on boar taint compounds

Immunocastration blocks the synthesis of testicular steroids, including androstenone, by interfering with the hypothalamic-pituitary-gonadal axis. Androstenone production is suppressed as a consequence of suppressed testicular function. The approach with immunocastration therefore does not only prevent androstenone formation selectively but also reduces the synthesis of anabolic steroids.

Immunocastration also reduces the level of the another boar taint compound, skatole [13, 17, 18, 40]. Even though skatole is produced in the intestine by microbial degradation of amino acid tryptophan and the immunocastration has no direct effect on skatole synthesis, reduction of skatole levels in immunocastrated pigs is related to hindered production of androstenone and oestrogens. Androstenone and 17-beta-oestradiol were identified as potential inhibitors of the expression and/or activity of major skatole-metabolising enzymes CYP2E1 [29, 30, 41] and CYP2A [42]. Indeed, activities of skatole-metabolising enzymes in the liver are higher in surgically and immunocastrated male pigs than the entire male pigs [43, 44]. Thus, in the absence of androstenone and 17-beta-oestradiol, the hepatic metabolism of skatole is not inhibited, and produced skatole metabolites are readily eliminated from the body.

Generally, for what regards the prevention of boar taint in pork, immunocastration is comparable to surgical castration as similar effects are achieved as in physical removing of the testes.

### 3.3. Effect of immunocastration on growth performance and carcass quality

Considering the entire fattening period (from the first vaccination until slaughter), meta-analysis of the effects of immunocastration on pigs' growth showed that immunocastrates grow faster than surgical castrates and entire males [45]. The explanation is that immunocastrates are physiologically entire males until the second (effective) vaccination, and therefore until then, they exploit boar-like growth potential. Following the second vaccination, rapid changes of the hormonal status start, characterised by the drop of the steroid levels [46]. Simultaneously, the concentrations of residual IGF-1 and somatotropin remain relatively high [47, 48], resulting in higher feed intake and growth rate of immunocastrates after the effective immunisation is reached. A study of Batorek et al. [49] revealed that, after effective immunisation, the immunocastrates increase fat tissue deposition at the expense of lower heat production, while protein deposition remains similar to entire males and different from surgical castrates, which deposit fat instead of protein (i.e. muscles). It is, however, important to take into consideration that these results were obtained with late immunocastration, where the first vaccination is performed at the start of the fattening period and the second vaccination very late, usually 4–6 weeks prior to slaughter (i.e. may not be the case for early immunisation). Studies dealing with early immunocastration are rare as such practice is not economically interesting. The level of fat deposition in immunocastrates has been related to the delay between the second vaccination and slaughter; and with longer delay, higher fat deposition is reported [33, 34, 50]. Although intramuscular fat deposits are regarded as favourable for meat sensory quality, the overall increase in body adiposity has negative impacts on economics of rearing (higher fatness leads to lower lean meat %, governing the carcass price). Summarising 30 studies, the meta-analysis of Batorek et al. [45] showed that immunocastrates exhibit thicker back fat than entire males, resulting in lower carcass lean meat percentage. On

the other hand, a comparison of immunocastrates with surgical castrates shows their advantages in terms of carcass quality (lower carcass fatness, heavier ham and shoulder). The way to control fat deposition in immunocastrates would be the manipulation of their diet after the second vaccination. Restricted feed intake [48] or energy dilution [51] improves carcass leanness due to lower fat deposition.

### 3.4. Effect of immunocastration on meat quality

Meta-analytical results [45, 52] show that immunocastrates and surgical castrates are very similar in regard to meat quality traits. On the other hand, compared to entire males (in addition to avoiding boar taint problem), immunocastrates exhibit superior meat quality as they have more intramuscular fat and more tender meat. Their fat is also more saturated, which is beneficial from the technological viewpoint. Besides that, unlike entire males, immunocastrates can be slaughtered at older age making their meat suitable for processing into dry-cured meat products, where raw material of specific quality is required. The available studies evaluating immunocastrates for dry-cured products show their similarity with surgical castrates in regard to meat and fat quality (including quantity and fatty acid composition) and are considered suitable for prolonged maturation process [36, 53–55]. A comparison of dry-cured hams originating from immunocastrates and entire males slaughtered at 130 kg [55] showed better aptitude of immunocastrates than entire males for long dry-curing maturation due to lower seasoning losses, lower salt intake and softer product with more intramuscular fat. However, it should be noted that fast changes of metabolism after the effective immunisation could reflect in changed protein turnover and consequently proteolytic activity of meat from immunocastrates, which is of relevance for long dry-curing process and would merit to be investigated for potential impact on product quality.

Due to the possible restauration of reproductive function and thus boar taint, triple vaccination protocol is considered in older, heavier pigs. Recent study comparing surgical castrates with double or triple vaccination [36] showed higher levels of boar taint compounds vaccinated only twice and slaughtered 14 weeks after the effective immunisation and concluded that three-dose immunocastration should be applied to meet the requirements for Italian PDO hams. The same study pointed out some indications of higher cathepsin activity than surgical castrates but only for immunocastrates vaccinated two times [36]. Similarly in the Iberian pigs [56, 57], the immunocastration with triple vaccination protocol has been found to be a suitable alternative as no major differences on carcass or technological and sensory meat quality were observed compared to surgically castrated females, whereas immunocastration of male pigs resulted in somewhat leaner carcasses with less intramuscular fat and lower tenderness than in surgical castrates.

Based on the studies, it can be concluded that the resemblance between immunocastrates and surgical castrates increases with the increase in elapsed time between the effective immunisation and slaughter. Depending on the need of pork industry, the protocol of vaccination can be adjusted (late or early vaccination, respectively). In summary, using immunocastration overcomes the drawbacks of pork production with entire males and is interesting for production systems with prolonged fattening (i.e. slaughter at higher age and weight) and extensive rearing systems.

### 3.5. Effect of immunocastration on animal welfare

Immunocastration itself, as a procedure, is considered a relatively welfare-friendly alternative. Compared to surgical castration without anaesthesia, it excludes acute pain associated with the procedure, the pain limited only to the needle insertion during application of the vaccine [10]. However, the administration in group-housing systems (or outdoor systems) may cause some practical difficulties that could trigger acute stress situations for pigs. The injection of the vaccine can also cause adverse reactions at the injection site, though these are most often reported as mild reactions [13, 58]. The injection of the vaccine is a systemic event leading to disturbance in the hormonal homeostasis of the animal; thus adverse effects could be expected in other tissues apart from the testes. One previous study suggested that immunisation against GnRH created tissue damages to the hypothalamus [59]. However, this was not confirmed in the later studies [60] likely due to improved vaccine formulation. The use of immunocastration on the other hand could overcome the mortality associated with surgical castration due to post-operation complications.

Until after the second administration of the vaccine, the immunocastrates are physiologically entire males, so compared to surgical castrates, they show male-like behaviour. This means more aggressive and mounting behaviour and higher number of skin lesions [61, 62]. However, after the second vaccination, aggressive and mounting behaviour is reduced to the level of surgically castrated pigs [63] in which standard production system happens in the period when aggressive behaviour would normally be intensified (i.e. at the age of 5–6 months). Soon after the effective immunisation, aggressive and mounting behaviour is reduced, while feeding behaviour becomes alike to surgical castrates [14, 19, 62]. Calmer behaviour is important for carcass quality because it is related to lower incidence of skin lesions, a consequence of fighting and mounting especially if unfamiliar pigs are mixed prior to slaughter (e.g. transport and lairage). Another aspect worth considering for the welfare of immunocastrates is related to their feeding. As their appetite is increased after the second vaccination, their feeding needs to be adapted to assure they are calm and satiety without negative effects on their body composition (energy dilution). Namely, restrictive feeding of immunocastrates showed similar level of aggression (i.e. incidence of carcass skin lesions) in restrictively fed immunocastrates as in entire males and higher as in ad libitum fed immunocastrates and surgical castrates [48].

## 4. Immunocastration and public acceptability

Despite the fact that the vaccine for immunocastration has been available in the European Union since 2009, its practical use is limited due to a generally low market acceptance [64]. Surveys with European stakeholders performed within PIGCAS project showed low prospects for immunocastration (surgical castration with anaesthesia/analgesia was preferred). It is also indicated that the main drawback of the immunocastration was the fear of consumers' acceptance [65]. However, opinion of consumers about immunocastration has not been thoroughly investigated, and they are mostly not well informed about boar taint and the methods used to prevent it [32, 66]. Consumers expect healthy, safe and tasty meat, which denotes that

boar taint represents an important concern for consumer acceptance [32]. Presently available studies about the consumer acceptability of the immunocastration show important differences across Europe. For Swiss consumers, the most acceptable alternative was surgical castration with anaesthesia/analgesia, while immunocastration was not favoured [67]. Swedish consumers expressed preference for meat from immunocastrates over the entire males and standard surgical castrates [68]. Belgian consumers, after being well informed on the existing alternatives, preferred immunocastration to surgical castration [69]. The same was observed for German consumers [70]. A survey with over 4000 consumers in France, Germany and the Netherlands [71] pointed out that the fear of negative consumer attitude towards immunocastration might be overestimated. Namely, in this survey immunocastration was acceptable for over 70% of the respondents. It is worth noting that a recent study [64] reported that Belgian farmers changed their attitude after having used different alternatives in a real life scenario and preferred entire males and immunocastration. For them, surgical castration with anaesthesia and/or analgesia was the least acceptable due to being the most demanding (labour intensive, costly and complex). In Belgium, immunocastration is practised by some farmers since 2011 because of retailers' demand [64]. Regarding other stakeholders, nongovernmental animal welfare organisations find immunocastration acceptable, although they prioritise rearing of entire males. According to PIGCAS project survey, the scientists perceive immunocastration as a better alternative to surgical castration with anaesthesia/analgesia due to being more practical and having benefits for animal welfare and economics [72]. Overall, it seems that the main obstacle for wider utilisation of the immunocastration resides in the fear of consumers and how they would accept this alternative. Other drawbacks expressed by stakeholders are related to the ease of use in group-housing or outdoor production systems and security at work (fear of self-vaccination).

## 5. Tools to assess effectiveness of immunocastration

Several studies have shown that the effect of immunocastration is very consistent among individuals. However, there are cases where nonresponders (0–3%) have been reported [39, 54, 73] in both small- and large-scale experiments. The reasons why some pigs escape the vaccination have not yet been sufficiently explained but may be ascribed to poor health status or malnutrition of the pig or the fact that some pigs are simply missed at physical vaccination in group-housing systems. This argues for the development of good tools to assess the effectiveness of immunocastration, e.g. at the slaughter line. Assessing the effectiveness of vaccination in live pigs basing on the observation of testes size or taking blood for hormonal analyses is practically difficult and economically unsustainable. Behavioural observations like high rates of mounting could also be warning signs used at the farm to detect possible nonresponders; however, this later is not very practical in large-scale farming systems. After the slaughter, a reliable method would be to chemically determine the level of boar taint compounds in fat tissue; however, for practical and economic reasons, simple, low-cost online methods are desired. One option would be to monitor the size and weight of the testes, which have been

shown to decrease significantly with successful immunocastration. However, as size/weight of testes is strongly related to pig's weight, it may not be a sufficiently reliable indicator of successful vaccination because of partly overlapping distributions between successfully immunocastrated and entire male pigs [17]. It was suggested that measuring seminal vesicle weight at slaughter line is more reliable to identify nonresponders [74]. A recent study [75] showed 100% success rate for prediction of nonresponders by combining the information on weight of all reproductive organs. In addition to morphological assessment of the size of reproductive organs at slaughter line, suspicious carcasses of immunocastrates could be additionally checked for boar taint by rapid methods involving the heating of fat tissue and sniffing.

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