Animal Health

Review

Current State of Immunological Castration of Male Pigs

Immunological castration of male pigs: current status

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ABSTRACT

Background: The downsides of fattening entire pigs is taint meat, aggressive behavior, and mating desire. Surgical castration reduces such behavior, and favors husbandry, while increasing weight, and improving meat quality. Additionally, boar taint is extinguished. However, this practice is controversial, since it causes injury, pain, and stress. Moreover, animals are put to risk due to infections, chronic inflammation, and post-operative complications. **Aim:** To examine the current state of immunocastration as a viable alternative to surgical castration of male pigs.
Development: Immunocastration is a safe commercially availablºe choice, which contributes to animal welfare, and it is viable for sustainable production of pigs. Moreover, it favors meat quality, cost-effectiveness, and environmental protection. However, its application is insufficient, except in Australia, Brazil, and New Zealand. Surgical castration predominates in most countries.
Conclusions: Immunocastration is a relatively recent technology, whose acceptance, introduction, and extension may generate uncertainties and resistance by different actors of the swine production chain. This practice calls for more technological discipline by farmers, in order to achieve the expected results. The extension of its use requires the approval of every interested part inside the production chain, besides markets that demand meat, as well as actors willing to sell it.
Key words: pigs, GnRH, immunocastration, peptide vaccines (*Source: MeSH*)

INTRODUCTION

Surgical castration of pigs has been in place since 4000-3000 B.C. (Zamaratskaia and Rasmussen, 2015). Pigs are usually castrated before 7 days of age (Kress, Millet, Labussière, Weiler, and Stefanski, 2019) to increase weight, improve the quality of meat, facilitate

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husbandry, and reduce promiscuous breeding. Also, the risk of boar taint decreases due to a reduction in the contents of androstenone (gonadal steroid) and skatole (from intestinal degradation of tryptophan) in the fatty tissue (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017).

However, this practice is controversial in terms of animal health and welfare (Kress *et al*., 2019). Suckling pigs respond to surgical castration with specific vocalizations (Von Borell *et al.,* 2009), and behaviors that indicate pain (Kress *et al*., 2019). Following castration, an increase is observed in the levels of cortisol, an adrenocorticotropic hormone (ACTH), and lactate, which are physiological indicators of stress (Prunier, Mournier, and Hay, 2005). Surgical castration causes injuries (Kress *et al*., 2019), and cannot be reversed. Animals are submitted to potential infections, chronic inflammation, and postoperative complications (Giersing, Ladewig, and Forkman, 2006), that lead to a delay in production and economic losses (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017). Besides, during the first week of life, mortality is higher in surgically castrated pigs than the in entire pigs (6.3% vs 3.6%) (Morales *et al*., 2017).

Surgically castrated pigs consume 10-15% more food to produce the same amount of meat, excreting almost 15% more nitrogen, compared to entire pigs. This leads to an increase in feeding costs and environmental impacts (Lundström, Matthews, and Haugen, 2009). Furthermore, the economic losses resulting from increased contents of fat in the carcass are higher (Bonneau and Weiler, 2019).

The World Organization of Animal Health (WOAH, 2019) recommends the application of this operation only when necessary, in order to minimize pain, stress or animal suffering. Among the choices offered by WOAH to strengthen animal welfare are the use of noncastrated or immunocastrated adult males, rather than surgically castrated animals. The European Declaration on surgical castration alternatives of pigs stipulates that, this practice would cease completely by 2018 (EC, 2010).

A minimally invasive and attractive alternative to surgical castration is active immunization against the gonadotropin releasing hormone (GnRH) from mammals, also named immunocastration or immunological castration (Zamaratskaia and Rasmussen, 2015). This procedure utilizes the immune system of pigs to generate anti-GnRH I antibodies, which temporarily suppress testicle functions, and prevent taint meat in the immunocastrated pigs (EMA, 2010).

This review article examines the current state of immunocastration as a viable alternative to surgical castration of male pigs.

DEVELOPMENT

GnRH from mammals or GnRH I

GnRH or luteinizing hormone releasing hormone (LHRH) is a critical molecule in the control of mammal reproduction (Whitlock, Postlethwait, and Ewer, 2019). Mammal GnRH, named GnRH I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2), was first isolated from pig hypothalamus (Matsuo, Baba, Nair, Arimura, and Schally, 1971). The size of this peptide is structurally preserved in all mammal species (10 amino acids), and the amine groups (pGlu-His-Trp-Ser), and carboxyl terminal (Pro-Gly.NH_2) (Millar, Pawson, Morgan, Rissman, and Lu, 2008).

GnRH I regulation of the hypothalamus-hypophyseal-gonadal axis

GnRH I is processed in the hypothalamic neurons through a precursor polypeptide, and transported by axons to tiny blood vessels in the outer area of median eminence. This peptide is released in the form of synchronized pulses of nervous terminals, in the hypophyseal portal system. The blood vessels that irrigate the anterior hypophysis allow the arrival of GnRH I at that point. In the hypophysis, GnRH I binds its receptors in the gonadotropic cells to stimulate the release of blood circulation of the follicle-stimulating hormone (FSH), and the luteinizing hormone (LH) (Whitlock, Postlethwait, and Ewer, 2019).

In pigs, LH stimulates synthesis and secretion of androgens (testosterone and dihydrotestosterone) by the leydig cells. Testosterone acts on the Sertoli cells, and it is necessary for spermatogenesis. Besides, testosterone has a negative feedback effect on LH secretion, through the suppression of GnRH I pulsating discharge by the hypothalamus. LH secretion is also controlled by other hormones, such as dopamine and prolactin (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017). FSH acts directly on the seminiferous tubules in the testicles (germinal cells and Sertoli cells), and triggers spermatogenesis, whereas, along with testosterone, it can maintain the production of sperm in adults. The Sertoli cells produce inhibin, which has a negative feedback effect on FSH secretion by the hypophysis. The growth hormone in pigs also stimulates the functional maturation of Sertoli cells. Thyroid hormones play a critical role in the normal development of testicles, both in the Sertoli cells and the leydig cells (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017). The interrelation triggered by the anterior hypophysis and the gonads, along with the action of the hypothalamus, unleashes this behavior (mating, aggression, etc.), traits, and sexual function, including libido in either sex.

GnRH I: a target molecule to manipulate the hypothalamic-hypophyseal gonadal axis

The manipulation of GnRH-I-centered hypothalamic-hypophyseal-gonadal axis is a potential tool to block gonadal function in male and female mammals, in order to delay puberty, prevent sexual and aggressive behaviors, and taint meat. Moreover, it is applied to produce fertility, treat diseases related to reproduction, and gonadal steroid dependent diseases, such as prostate cancer, breast cancer, and endometriosis (Rosenfield and Pizzutto, 2018).

The objective of immunocastration is to deactivate testicle functions, and affect male behavior, by neutralizing the hypothalamic-hypophyseal-gonadal axis. Active immunization against GnRH I includes the injection of a GnRH I analogue, which is conjugated to a foreign protein, and combined with an adjuvant (Heegaard, Fang, and Jungersen, 2016) to initiate the formation of antibodies that neutralize the endogenous action of GnRH I (Zamaratskaia and Rasmussen, 2015). GnRH I is a heptane and a self-antigen. Some of the strategies to generate antibodies against this molecule include the generation of one or several copies of GnRH I-analogue peptides, which cannot be seen as self by the immune system. These peptides must couple to carrier protein, like tetanus toxoid, diphtheria toxoid or its synthetic T-helper epitopes, key hole limpet hemocyanin (KLH), ovalbumin (OVA) (Gupta and Minhas, 2017), and bovine serum albumin (BSA).

Commercial vaccines for immunocastration of pigs

Today, Improvac® and its globally related brands: Improvest® (USA and Canada), Vivax®, and Innosure®, are available for immunocastration of pigs (Kress *et al*., 2019). This vaccine was developed in Australia (CSL Limited, Parkville, Victoria, Australia), and is currently manufactured by Zoetis Inc. (previously by Pfizer Ltd.). Its use was approved in Australia and New Zealand, in 1998, to prevent boar taint meat.

The active pharmaceutical ingredient of Improvac® is an incomplete analogue peptide of GnRH I, which is conjugated to diphtheria toxoid, and adjuvated in diethylaminoethyl-dextran (DEAE) (McNamara, 2014).

A similar vaccine, Ceva Valora®, which contains three synthetic peptides as immunogens, is sold by Ceva Animal Health. In this vaccine, GnRH is covalently bound to sequences of T-helper cells at the N-terminal end. However, this vaccine is not available commercially in Europe (EC, 2019).

Effects of immunocastration in pigs

Vaccination with Improvac®

The pigs are given two shots of Improvac®, subcutaneously, in the neck, just behind the ear. The first dose is administered after eight weeks of age, and the second, four weeks following the first dose, minimum, and four to six weeks before sacrifice (EC, 2019). Provided the two doses of Improvac® are properly administered by the technician, almost 100% of animals respond positively, and barely 0.3% requires a third shot. The latter results from the no application of the vaccine (EC, 2019). A recent study done by Kress *et al*. (2020) concluded that careful application of this vaccine ensures reliable results, even under different pig lodging conditions.

Pigs, which are fattened for a longer period (sacrificed at 14 months), might require three doses of the vaccine to ensure efficient activation of endogenous GnRH, and the elimination of boar taint. The third dose is applied 10 weeks after the second dose, between 4 and 6 weeks prior the date of sacrifice (EC, 2019).

Immunocastration is performed during the finishing period to make use of all the growth potential of entire male pigs, until the second shot (Zamaratskaia and Rasmussen, 2015). The first dose prepares the immunological system of the pig, but causes no relevant physiological changes in the animal. The second dose stimulates the immune system to produce specific antibodies that suppress testicle function. From a welfare perspective, the administration of the vaccine is less harmful to the pig, compared to surgical castration without anesthetics or analgesics (Nautrup, Vlaenderen, Aldaz, and Mah, 2018).

Levels of testosterone and skatol

The effects of Improvac® are reversible. The concentrations of androstenone and skatol drop significantly between the second and third weeks following the second dose, and remain like that for approximately 10 weeks following the second shot (EC, 2019). Cosequently, the pigs immunized with Improvac® lose the effect of taint meat.

The production of androstenone is suppressed as a result of testicle atrophy. The reduction of skatol is more likely to happen due to an increase in the metabolism of the liver and further purification in the absence of testicle steroids, particularly androstenone and estrogens (Zamaratskaia, and Rasmussen, 2015).

Characteristics of reproductive organs

At sacrifice, vaccination with Improvac® causes a reduction in the weight of testicles (between 16 and over 90%), the bulbourethral glands (between 50 and over 90%), and seminal vesicles (between 36 and over 90%, (Škrlep *et al.*, 2010; Einarsson *et al.*, 2011; Stupka *et al.*, 2017; Sládek, *et al.*, 2018; Kress *et al*., 2020). The differences reported in the weights of reproductive organs are linked to the initial moment, the second shot with Improvac®, and the time elapsed until sacrifice (Nautrup *et al.*, 2018; Zoels *et al*., 2020).

The seminal vesicles, compared to testicles, bulbourethral glands, and prostates, undergo a greater weight reduction in immunocastrated pigs. Hence, Bonneau (2010) suggests checking the effectiveness of immunocastration in pigs, according to the size of seminal vesicles, not the testicles.

Although observation and measurement of testicle size and weight are not effective methods to determine the efficacy of vaccination with Improvac®, they are not effective due to variations in pig testicle size (Čandek-Potokar, Prevolnik, and Škrlep, 2014; EC, 2019). The size of the bulbourethral gland may be a good indicator of success in immunocastration (Čandek-Potokar, Prevolnik, and Škrlep, 2014). However, it is very small and could be damaged during animal sacrifice. The safest method to demonstrate the effectiveness of immunocastration is by determining testosterone, because its levels tend to correlate with androtestone levels.

Productive behavior

The results of this meta-analysis performed by Nautrup *et al.* (2018), which includes 78 published studies, confirm improved growth performance in pigs immunocastrated with Improvac®, compared to the physically-castrated pigs, and entire pigs. The immunocastrated animals gained more weight on a daily basis, along with more favorable food conversion, and a similar risk of having taint meat, compared to castrated pigs. These authors noted that the weight of immunocastrated pigs at sacrifice is greater than the weight of entire pigs (approximately 3.0 kg, $P < 0.0001$), and physically castrated pigs (approximately 2.0 kg, $P = 0.018$). Nautrup *et al.* (2018) concluded that the optimum growth of immunocastrated pigs can only be acquired through the administration of a customized diet. Moreover, these productive results depend on the time lapse between the second vaccination and sacrifice.

Pig welfare, behavior, and health

The pigs immunocastrated with Improvac® behave like entire pigs (Dunshea *et al.*, 2013), until after the administration of the second vaccine, and therefore, have an increased aggressive behavior. The aggressive and sexual behaviors are important indicators of animal welfare, since high levels of mating and aggression cause stress, fear, and injuries to pigs (Rydhmer *et al.*, 2006).

Between 4 and 6 weeks after the second shot, the sexual and aggressive behaviors (mating, fighting, pushing, and head and tail movements) are significantly reduced to the same level of surgically castrated pigs (Škrlep, Batorek-Lukač, Prevolnik-Povše, and Čandek-Potokar, 2014; Karaconji, Lloyd, Campbell, Meaney, and Ahern, 2015), due to the low levels of testosterone and estrogens. Hence, immunocastrated pigs are less prone to suffer cutaneous injuries, compared to entire pigs, at sacrifice (Rydhmer, Lundström, and Andersson, 2010).

Immunocastration also reduces the frequency and severity of injuries on the penis, compared to entire pigs of the same age and weight (Reiter, Zöls, Ritzmann, Stefanski and Weiler, 2017; Zoels *et al*., 2020). All these effects increase animal welfare, by preventing painful surgical castration and the risk of infections. Besides, it reduces stress, fear, and injuries (Kress *et al*., 2019; Weiler and Bonneau, 2019; Zoels *et al*., 2020).

The most frequently observed adverse reactions caused by Improvac® are swelling at the site of injection, which is gradually reduced, though it may last for over six weeks in 20-30% of animals. Additionally, a transient rise in rectal temperature (0.5 °C) is observed during the next 24 hours following vaccination (EMA, 2009). These reactions can be prevented or minimized if vaccination is performed by skilled personnel, according to the specifications of the manufacturer (Kress *et al*., 2019).

Environmental impact

Improvac® does not contain chemical products or microbiological agents that could damage the environment. Moreover, the vaccinated pigs do not excrete metabolites from Improvac® (EMA, 2010).

The improvement observed in food conversion implies that the pigs vaccinated with Improvac® produce fewer feces throughout the year than the surgically castrated pigs (De Moraes *et al.*, 2013). This cuts down the environmental impact of swine production, by decreasing the emission of greenhouse gases, and the amount of nitrogen and phosphorus from feces. The pigs castrated with Improvac® have a global heating potential of 3.7% and 5.0% lower per live weight and meat kg, respectively than the surgically castrated pigs (De Moraes *et al.*, 2013). Pig immunocastration contributes to sustainable swine production (Kress *et al*., 2019; Morgan *et al*., 2019).

Economic advantages

In Europe, a dose of Improvac® costs 1.4-1.5 Euros (EC, 2019). The additional vaccination costs are compensated with more income, thanks to increases in production in areas and years (Kress *et al*., 2019).

The yield data collected from 12 studies done in the USA suggest that vaccination with Improvest® offers a potential advantage to farmers, consisting of $10.32 USD per animal. The expected yields for meat packers are $5.04 USD per carcass, which is associated to an increase of meager meat. In a recent study, Morgan *et al.* (2019) concluded that the proposals of animal welfare-friendly alternatives, including immunocastration of pigs, may be economically advantageous for the American swine market.

Global situation in terms of pig immunocastration

In 2013, it was estimated that (De Moraes *et al*., 2013) 95% of pigs in the world had undergone surgical castration to take away boar taint. Between 2015 and 2018, the number of immunocastrated pigs doubled around the world (Kress *et al*., 2019), which demonstrates the growing use of this technology.

In 2010, the European Declaration on alternatives to surgical castration of pigs was established. This declaration stipulates that by January 1st, 2012, surgical castration of pigs would be performed with the utilization of analgesics and/or anesthetics. Additionally, it called for a complete elimination of this practice by 2018 (EC, 2010). However, most European countries still perform surgical castration of pigs without analgesics or anesthetics (Backus, Higuera, Juul, Nalon, and de Briyne, 2018).

A study developed by De Briyne, Berg, Blaha, and Temple (2016), in 24 European countries acknowledges that only 2.7% of pigs are immunocastrated; 36% are not castrated; and 61% are surgically castrated (5% using analgesics and anesthetics, 41% only using analgesics, and 54% using no analgesic or anesthetics). Slovakia and Belgium have the highest estimated percents of immunocastrated pigs (10 and 18%, respectively). Eighteen European countries utilize surgical castration to 80% or more of their pigs. Spain, Norway, the Czech Republic, Romania, and Sweden reported a slight increase in the number of immunocastrated pigs in the last 3-5 years.

Entire pig production for meat consumption is relatively new to most European countries, except the United Kingdom, Ireland, Spain, and Portugal, whose figures are between 80 and 100% (Table 1). In recent years, the production of these animals has increased in Germany, Belgium, France, and the Netherlands (Backus *et al*., 2018).

Table 1. Percent of entire, immunocastrated, and surgically castrated pigs sold in 24 European countries (Backus *et al*., 2018).

Country	Entire pigs (%)	Immunocastrated (%)	Surgically castrated (%)	Swine population x 1000, heads
Austria	5	0	95	2846
Germany	20	<1	80	27 600
Belgium	8	15	80	6351
Czech Republic	5	5	90	1548
Denmark	<2	0	>97	12 402
Slovakia	0	10	90	637
Slovenia	1	0	99	288
Spain	80	5	15	28 500
Estonia	0	0	100	359
Finland	4	0	96	1258
France	22	<0.1	78	11 835
Hungary	1	0	99	2935
Ireland	100	0	0	1468
Iceland	0	0	99	36
Italy	2	5	93	8561
Latvia	0	0	100	368
Luxembourg	1	0	99	90
Norway	<1	6	94	1644
The Netherlands	65	0	35	12 013
Portugal	85	2.5	12.5	2014
The United Kingdom	98	<1	2	4383
Romania	0	5	95	5180
Sweden	1	9	90	1354
Switzerland	5	2.5	92.5	1573
Total	**34.0 %**	**2.8%**	**63.0%**	

Backus *et al*. (2018) concluded that, according to European experts, 63% of pigs are castrated, most without anesthetics or analgesics, whereas 34% are not castrated, and only 2.8% are immunocastrated (Table 1). These results are similar to De Briyne *et al*. (2016). In a study conducted by Backus *et al*. (2018), remarkable figures are still observed among European countries, in terms of swine production. Most of them still using surgical castration, which fail to comply with the European Declaration on alternatives to surgical castration of pigs. The same situation continues to be the same today (Kress *et al*., 2019).

In short, several European countries have begun to replace surgical castration by entire and/or immunocastrated pig production. However, nearly all still undergo surgical castration.

In the USA, extensive immunocastration of pigs has not been implemented (Rueff, Mellencamp, and Pantoja, 2019). In Australia, Brazil, and New Zealand, immunocastration is more widespread. In Australia and Brazil, over 50% of pigs are immunocastrated (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017; Mancini, Menozzi, and Arfini, 2017; D'Souza, Hewitt, and van Barneveld, 2018). The ban placed on entire pig slaughtering in Brazil (Decree No. 9,013, Brazil), among other factors, favors greater implementation of immunocastration.

Why immunocastration is not very outspread

Improvac® has been approved in more than 60 countries (Zamaratskaia and Rasmussen, 2015), including Brazil, the European Union (2009), Japan (2010), China (2010), and the USA. (2011). However, its practical application is still very limited.

The application of this vaccine is infrequent, since the perception of immunocastration is very heterogeneous among countries (Mancini, Menozzi, and Arfini, 2017). Besides, the acceptance of this procedure within the swine market is low (Aluwé, Tuyttens, and Millet, 2015a; Kress *et al*., 2019). In turn, swine farmers are not so confident in that this alternative can prevent taint meat, and doubt its profitability (Aluwé, Vanhonacker, Millet, and Tuyttens, 2015b). Besides, they are concerned about the possible feasibility of using immunocastration in different productive systems (Čandek-Potokar, Škrlep, and Zamaratskaia, 2017). In that study, the main concern of farmers was the risk of accidental self-injection. These accidents may cause the same effects to the technicians who use Improvac®, observed in the pigs, and their consequences are more serious if other accidental doses occur. However, the incidence of accidental self-injection after the application of more than 7 million doses of Improvac® is 0.00004% (EMA, 2010). To minimize the risk, this vaccine can only be applied with the safety gear provided by the manufacturer, and by properly trained personnel.

Another inconvenient to more extended application of immunocastration is linked to consumer acceptance fears (Kallas *et al*., 2013; Škrlep, *et al*., 2014; Aluwé *et al*., 2015b). The European consumers were more cautious and conservative. However, studies done in Switzerland, Norway, and Belgium indicate that these concerns may be overestimated, and that properly informed consumers do accept it (Čandek-Potokar, Škrlep, and Batorek Lukac, 2015).

Furthermore, most consumers are not well informed about taint meat, the preventive choices (immunocastration), and its benefits (Kallas *et al*., 2013; Škrlep *et al*., 2014; Mancini, Menozzi, and Arfini, 2017). However, consumers who are knowledgeable of its benefits show high acceptance and preference for it over physical castration, though the meat from immunocastrated pigs is costlier (Zamaratskaia *et al*., 2008; Vanhonacker and Verbeke, 2011).

Surveys conducted by Di Pasquale *et al.* (2019) showed that most Italian consumers have a positive perception of immunocastration, with a relatively low level of risk perception, and a will to pay more for the meat. The information available about this procedure does not affect consumer perception.

In a study, Mancini, Menozzi, and Arfini (2017) concluded that most consumers do not rely on the safety of meat from immunocastrated pigs. The European Medication Agency (EMA) reported that the meat from pigs vaccinated with Improvac® is completely harmless to human consumption (EMA, 2010). This vaccine does not activate when ingested; it does not leave traces in the meat, which might affect human health, and does not show any hormonal activity. In turn, the Food and Drug Administration of the USA (FDA) has determined that the meat from pigs vaccinated with Improvest® is safe to eat, since it does not contain residues that can affect human health (FDA, 2011).

CONCLUSIONS

Immunocastration is a commercially feasible safe choice, which is friendly to animal welfare, and viable for sustainable production of pigs. It favors meat quality, cost-effectiveness, and environmental protection. However, its application is insufficient, and surgical castration predominates in most countries. Immunocastration is a new practice whose acceptance, introduction, and extension may generate uncertainties, and resistance by different actors of the swine production chain. This new practice imposes changes in the way farmers interact with rearing, and it demands higher technological discipline in order to achieve the expected benefits. The extension of its use requires approval by interested parts within the production chain, as well as compensation to farmers for additional purchasing costs and administration of the vaccine, markets that can assume the supply, and the actors willing to sell it.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.