

ЕКОЛОГИЯ И КАЧЕСТВО НА ЖИВОТИНСКАТА ПРОДУКЦИЯ**ALTERNATIVES TO SURGICAL CASTRATION OF PIGS⁺**MARJETA ČANDEK-POTOKAR^{1,2}, NINA BATOREK LUKAČ¹¹Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia²University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia

Castration or gonadectomy is a surgical procedure performed on male pigs for centuries in which testis and epididymis are physically removed from scrotal sack, without anaesthesia or analgesia, in the first days of life (**Council Directive**, 2008), mainly to get calmer and fatter pigs that do not exhibit boar taint and to prevent sexual behaviour in group housing (**EFSA**, 2004). For conventional production in the European Union (EU), castration is performed on 80 to 100% of male pigs (with the exception of Ireland 0%, United Kingdom 2.1%, Portugal 11.2%, Spain 33.3%, Cyprus 39% and Greece 75.6%), 97% of which are castrated without the use of anaesthesia (**EFSA**, 2004). Although generally used, this form of castration has been recently criticized as being painful for the piglets (**McGlone et al.**, 1993 and **Prunier et al.**, 2006) and may be considered even unnecessary in modern pig production with the emergence of new, alternative techniques, especially if pigs are raised for meat consumption where raising entire males (EM) would be more efficient (**Bonneau**, 1998 and **Trefan et al.**, 2013). Moreover, the payment system of pig carcasses in most European countries rewards leanness, which gives EM additional advantage over surgical castrates (SC), provided that the carcasses are not being de-valued due to the presence of boar taint. Thus, taking into account the welfare as well as the economic aspects it has been decided at the EU level that pig producers will voluntarily stop castrating until 2018 (http://ec.europa.eu/food/animal/welfare/farm/initiatives_en.htm; <http://boars2018.com>) and at the same time, intensive research of alternative methods to surgical castration, diagnostic methods for boar taint analysis and harmonization of analytical methods is being supported.

Boar taint

Boar taint refers to the offensive odour or taste, evident during cooking or eating of meat or meat products from some, mostly EM pigs (**Bonneau et al.**, 1992). It makes pork undesirable for sensitive consumers (**Weiler et al.**, 2000). The substances that are considered the primary cause of boar taint are androstenone, a male pheromone, (**Patterson**, 1968) and skatole, a by-product of tryptophan breakdown in large intestine (**Vold**, 1970 and **Walstra and Maarse**, 1970). Androstenone (5 α -androst-16-ene-3-one) is an anabolic steroid, without androgenic effect (**Claus et al.**, 1970), but with pronounced urine-like odour and flavour. It is

produced by the Leydig cells in the testis of sexually mature male pigs (**Gower**, 1972). Due to its lipophilic character, it accumulates in adipose tissue in much higher amounts than other sex hormones (**Pearce et al.**, 1988). Part of androstenone is stored in salivary glands, bound to specific binding protein pheromaxin (**Booth and White**, 1988), and after being released in the saliva, it serves as a pheromone to promote sexual behaviour in female pigs (**Pearce et al.**, 1988). Unlike androstenone, skatole (3-methyl-indole) does not seem to have a biological function in pig. It is a by-product of amino acid L-tryptophan breakdown by bacterial microflora in large intestine of pig. Thus the amount of skatole produced is primarily regulated by the availability of L-tryptophan which mainly originates from gut-mucosa cell debris (**Claus et al.**, 1994 and **Claus and Raab**, 1999) and the activity of intestinal bacteria, especially *Escherichia coli*, *Lactobacillus* sp., *Clostridium* sp. (**Jensen et al.**, 1995a) and *Olsenella* sp. (**Jensen**, 2012). Skatole that is produced in the colon is then partly excreted with faeces and partly absorbed through intestinal wall and released into the bloodstream. The metabolism and degradation of skatole in liver is inhibited by steroid hormones (**Doran et al.**, 2002), and due to its lipophilic nature, it also accumulates in adipose tissue. The odour of skatole is described as faecal like or sometimes naphthalene like and can be detected by the vast majority of people (**Weiler et al.**, 2000). On the contrary, androstenone has a large variation in consumer's sensitivity, as some people can detect it in very low concentrations, whereas approximately 25% are anosmic to it (**Wysocki and Beauchamp**, 1984 and **Claus et al.**, 1994).

Alternatives to surgical castration of pigs

Possible alternatives to surgical castration with explanations of their way of action and the positive and negative aspects of their use and application are summarized in Table 1. Among them the alternatives that are the most promising are genetic selection for reduced boar taint, sex preselection (by sperm sorting) and rearing only females, immunocastration, surgical castration with pain relief (the use of analgesia/anaesthesia) and rearing of EM. Although one of the easiest ways to neutralize male fertility would be using substances that lead to local destruction of testicular tissue (e.g. formaldehyde, zinc or silver salt, acetic acid), because they are easy to administer, not expensive and

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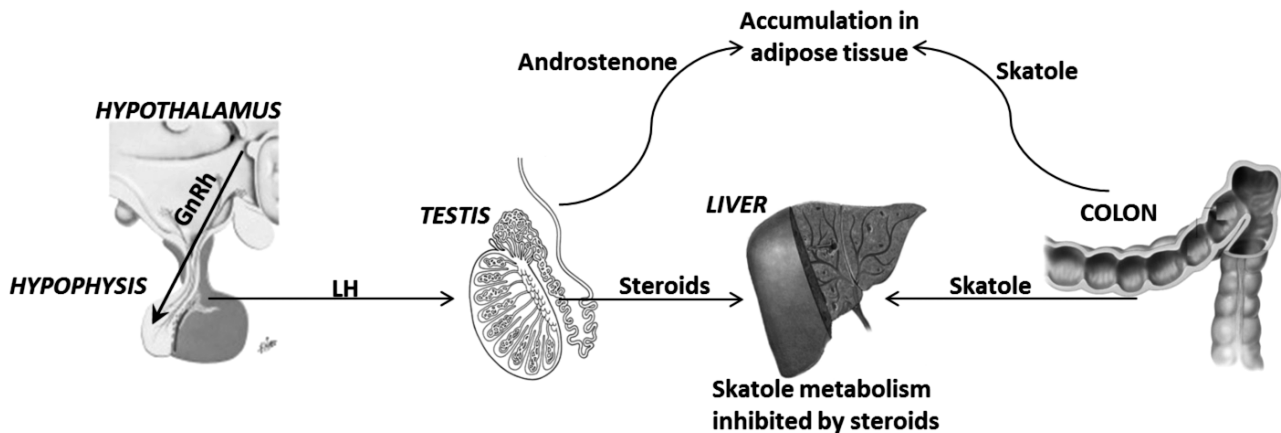


Fig. 1. Relationships between the hypothalamic-pituitary-gonadal axis, androstenone production in testes and skatole formation from tryptophan in intestine and their metabolism in liver. In boar, the production of testicular steroids, including androstenone, primarily a male pheromone, inhibits hepatic metabolism of skatole. Androstenone and skatole are therefore, due to their lipophilic character, deposited in adipose tissue.

cause no haemorrhages, the use of such substances causes inflammatory reaction, leading to swelling, necrosis and subsequently pain (Fahim, 1994 and EFSA, 2004) and is therefore unacceptable from a welfare point of view. Also the use of endogenous hormones (gonadotropin releasing hormone – GnRH agonists or antagonists) in order to neutralize GnRH provides no alternative to surgical castration in the EU, because their use is strictly limited to medical purposes, mostly for the treatment of reproductive disorders (Council Directive, 1996 and Stephany, 2001). Thus, from a practical point of view only immunocastration, rearing of EM and castration with pain relief are acceptable alternatives in the EU, which can be applied immediately, whereas sex preselection by semen sorting and genetic selection for low androstenone level are long term, but sustainable and welfare friendly solutions (EFSA, 2004).

Castration with pain relief

Castration under anaesthesia or/and with the use of analgesia is basically the same surgical procedure as is the one being performed nowadays, except that with the application of pharmacological agents to the piglets prior or/and during the operation the pain is omitted or reduced. Hence, the use of analgesia/anaesthesia improves animal welfare of piglets significantly, but it generates extra costs on pig farms which depend on the substances being used and the form of their application. The costs increase significantly if the anaesthesia cannot be administered by farmers and when the veterinarian has to be called (de Roest et al., 2009). There are numerous possibilities of reducing or avoiding pain caused by castration that can be in general divided in local or general anaesthesia and analgesia alone, however they all require additional handling of the piglets inducing additional stress and greatly extending the time required for performing the castration (EFSA, 2004). The method of choice should result in a significant reduction or elimination of pain, not only sedation, discomfort and stress

for the piglets; moreover the substance has to be approved for the use in pigs reared for meat consumption (under Council Regulation No 2377/90), the dose of the substance has to be consistently adhered, because the toxic or even lethal dose can easily be exceeded in young piglets, and it would be preferable that it can be administered by the farmer alone, which significantly narrows down the available options. The remaining options, giving satisfactory results in term of pain relief, registered for use in animals raised for meat consumption, which might be used without additional equipment on the farm are:

- use of pre-operative analgesia alone – intra-muscular administration of non-steroidal anti-inflammatory drug (e.g. Ketoprofen, Meloxicam);
- use of combination of pre-operative analgesia and local anaesthesia – intra-muscular administration of non-steroidal anti-inflammatory drug and intratesticular or intrafunicular administration of 1% lidocaine with adrenalin.

Other options all have some drawbacks; the application of anaesthetic mixture via nasal spray is insufficient to induce adequate depth of anaesthesia (Axiak et al., 2007), general anaesthesia by injection was insufficient in significant number of piglets and has to be administered by veterinarian (Leeb et al., 2008), general anaesthesia by inhalation (isoflurane or sevoflurane – none is currently registered for the use in pigs) requires special equipment, veterinarians' assistance plus application of analgesic (e.g. non-steroidal anti-inflammatory drug), because it does not provide sufficient pain relief when used alone (Kupper et al., 2008; Schulz, 2007; Hodgson, 2007 and EFSA, 2004). There is a thin line between lethal and anaesthetic dose and also inhalation anaesthesia and CO₂ are aversive prior to the loss of consciousness (EFSA, 2004). Norway and Switzerland have already prohibited surgical castration without pain relief and have introduced alternative methods – the castration is performed by veterinarians and anaesthesia is mandatory (EFSA, 2004).

Table 1. Summary of alternative methods to surgical castration with explanations of their way of action and its pro et contra

Alternative	Way of action	Pro	Contra	Reference		
Raising entire male pigs	Detection of boar taint	Trained panellists on slaughter line	Cooking test	Detecting tainted carcasses regardless to their source	Useful only on small number of animals, subjective method	Bundesanzeiger , 2007
		Soldering iron applied to the exposed backfat of the carcass	On line method for boar taint detection	On line method for boar taint detection	Possibility of false positive or false negative results, subjective	Jarmoluk et al. , 1970
	Skatole analysis on slaughter line	Spectrophotometric method	Successful and relatively fast (180 samples/hour)	Successful and relatively fast (180 samples/hour)	Androstenone is not detected	Mortensen and Sorensen , 1984
	Electronic nose	Chemical electronic sensors which measure androstenone and skatole	Measures both substances at one occasion, all tainted carcasses are detected	Measures both substances at one occasion, all tainted carcasses are detected	Only experimental, 16% false positive results	Annor-Frempong et al. , 1998 Ampuero and Bee , 2006
	Mass spectrometry	Pyrolysis-mass spectrometry	High classification rates	High classification rates	Experimental	Ampuero and Bee , 2006 Ampuero et al. , 2008
Slaughter at lower weight		Slaughter of pigs below 90 kg	Reduces the incidence of tainted carcasses, higher % lean meat, better feed efficiency	Reduces the incidence of tainted carcasses, higher % lean meat, better feed efficiency	Requires detection of boar taint on slaughterline, lighter carcasses are less profitable, meat is unsuitable for dry-meat products	Bonneau , 1987 Zamaratskaia et al. , 2005 Aldal et al. , 2005 Chen et al. , 2007
Controlling boar taint through nutrition and rearing conditions		Farrow-to-finish system, feeding diets rich in indigestible carbohydrates, clean environment, ideal temperature and max. ventilation rate	Reduced skatole level, no adverse effect on growth	Reduced skatole level, no adverse effect on growth	No effect on androstenone level	Hansen et al. , 1994 Jensen et al. , 1995 Rideaut et al. , 2004 Zamaratskaia et al. , 2005 Andersson et al. , 2005
Blocking reproductive axis	Targeting GnRH	GnRH agonists	Negative feedback of GnRH agonist on GnRH synthesis	Negative feedback of GnRH agonist on GnRH synthesis	Short-term effect, hormonal treatment is expensive and not permitted in EU	Reid et al. , 1996 Sinclair et al. , 2001 Brussow et al. , 2011 Ziecik et al. , 1989
	GnRH antagonists	Permanent occupation of GnRH receptors by antagonist	Immunization effect in 24 h	Immunization effect in 24 h	Short-term effect, large amounts of antisera and frequent administration is required	Van der Lende et al. , 1993
	Passive immunization	Application of anti GnRH serum				
	GnRH targeted toxins	Destruction of GnRH receptors bearing cells by GnRH coupled to cytotoxins	Single treatment reduces testosterone to 0 for approximately 20 weeks	Single treatment reduces testosterone to 0 for approximately 20 weeks	Experimental, not used on pigs	Sabeur et al. , 2003 Harrison et al. , 2004

Alternative	Way of action	Pro	Contra	Reference
Targeting androstenone	Immunization against 5 α -androst-16-en-3-one	Reduction of androstenone conc. in adipose tissue, advantages of male type performance, carcass composition is preserved	Experimental	Williamson et al., 1985
Surgical castration	With local anaesthesia	Intrafunicular and/or intratesticular application of local anaesthetic (commonly lidocaine with adrenalin)	No economic benefits, additional cost, requires veterinary assistance, pain is not eliminated	EFSA, 2004 Prunier et al., 2006 Fredricksen and Nafstad, 2006
	With general anaesthesia	Intramuscular application of anaesthetic mixture prior castration	Long recovery period, losses are 3-5 %, insufficient depth of anaesthesia, additional cost and veterinary assistance	Lahrman et al., 2006 Leeb et al., 2008 Zankl, 2007
	By inhalation	Anaesthesia with isoflurane by mask	Expensive, time consuming, requires veterinarian assistance, high mortality of piglets	Shulz, 2007 Kupper et al., 2008
	By injection	CO2 inhalation	Exposure to CO2 is aversive until loss of consciousness, possibility of overdose	Svendsersen et al., 2005 Kluivers-Poodt et al., 2007
	By nasal spray	Administration of anaesthetic mixture (Ketamin, Azeperone and Climazolame) via nasal spray	Insufficient depth of anaesthesia, possibility of overdose	Axiak et al., 2007
With analgesia	Meloxicam/ Metamizol / Ketoprofen	Application of analgesic prior castration	Additional cost and veterinary assistance is required	Heinritzi et al., 2006 Zöls et al., 2006
Chemical castration	Formaldehyde, zinc or silver salt, acetic acid	Local destruction of testicular tissue	Painful inflammatory reaction	Fahim, 1994 Giri et al., 2002

Alternative	Way of action	Pro	Contra	Reference
Sperm sexing Sperm Sexing Technology	Sex preselection (producing female litters) by flow-cytometry cell sorting	Females do not exhibit boar taint	To slow for commercial use, requires intrauterine insemination, 85-95% purity, less efficient production and fatter carcasses than boars	Johnson et al., 1989 Johnson, 2000
Semen sexing kit (high volume sperm sexing technology)	Binding x-bearing cells by Ig, filtration deagglutination of X-bearing cells	Faster than BSST, produces high-volume of x-bearing sperm (good potential for commercial use)	Experimental, 70% purity	ter Beek, 2007
Genetic selection	Identifying genes responsible for low androstenone production or high androstenone and skatole metabolism and selection of such animals	It would be the most welfare friendly method, which would not require castration or boar taint detection on line	It is a long term solution, still in development	Willeke et al., 1987 Bonneau et al., 1987 Willeke and Pirschner, 1989 Sellier et al., 2000 Moe et al., 2007

Raising entire males

Raising EM (boars) is a common practice in some EU countries (United Kingdom, Ireland, Spain, Portugal) and is gradually being introduced by some pig chains in the Netherlands, Germany, Belgium and France. Compared with SC rearing of EM it is more cost effective (no cost and wound infections due to castration, better FCR and better carcass conformation – higher meat percentage; **Bonneau, 1998**). However, this type of farming is more demanding for the farmer and more stressful for the animals (more aggression and sexual behaviour as they mature; **Prunier et al., 2006**); moreover there is a possibility for occurrence of boar taint in meat and meat products from EM (rejected by majority of consumers; **Malmfors and Lundström, 1983** and **Weiler et al., 2000**), lower meat quality (decreased tenderness and lower water holding capacity, reduced intramuscular fatness; **Babol and Squires, 1995** and **Trefan et al., 2013**) and subsequently decreased suitability for processing into high quality meat products (**Diestre et al., 1990** and **Banon et al., 2003**). Because the occurrence of boar taint at commercial slaughter weights is very variable, ranging from 10 to 75% (**EFSA, 2004**), depending on breed, slaughter weight, rearing and management conditions, the production of EM requires screening and sorting of carcasses on the slaughter line. Currently there are only subjective methods that can be applied (cooking test and use of soldering iron applied on exposed backfat of the carcass; **Bundesanzeiger, 2007** and **Jarmoluk et al., 1970**) that require trained panellist, as the objective methods that would give immediate or quick result are either still experimental (electronic nose and mass spectrometry; **Annor-Frempong et al., 1998** and **Ampuero and Bee, 2006**) or detect only one component of boar taint (spectrophotometric method for skatole analysis and ELISA kit for androstenone analysis; **Mortensen and Sornsen, 1984** and **Walstra et al., 1999**). In recent years, several strategies to prevent or reduce occurrence of boar taint meat have been developed. They include the use of special feeding strategies (different non-starch polysaccharides and additives; **Albrecht, 2011**), slaughter at a lower weight (prior sexual maturity; **Bonneau, 1987**) and improving rearing conditions (farrow-to-finish system, clean environment, maximal ventilation rate and temperature in termoneutral zone; **Salmon and Edwards, 2006**, **Hansen et al., 1994** and **Andersson et al., 2005**). Thus, if detection of the tainted meat on the slaughter line and improved processing of tainted meat is provided, the rearing of EM can easily be applied.

Immunocastration

Immunocastration uses natural immune system of the pig for the formation of specific antibodies that bind and neutralize GnRH. It consists of two vaccinations, as a result hypothalamic-pituitary-gonadal axis is blocked and testes growth and sexual steroids synthesis are effectively inhibited (see Fig. 2). Physiologically the immunocastration becomes effective in a week following second vaccination (V2; **Claus et al., 2007**), therefore in case of late revaccination during the month preceding slaughter, growth characteristics

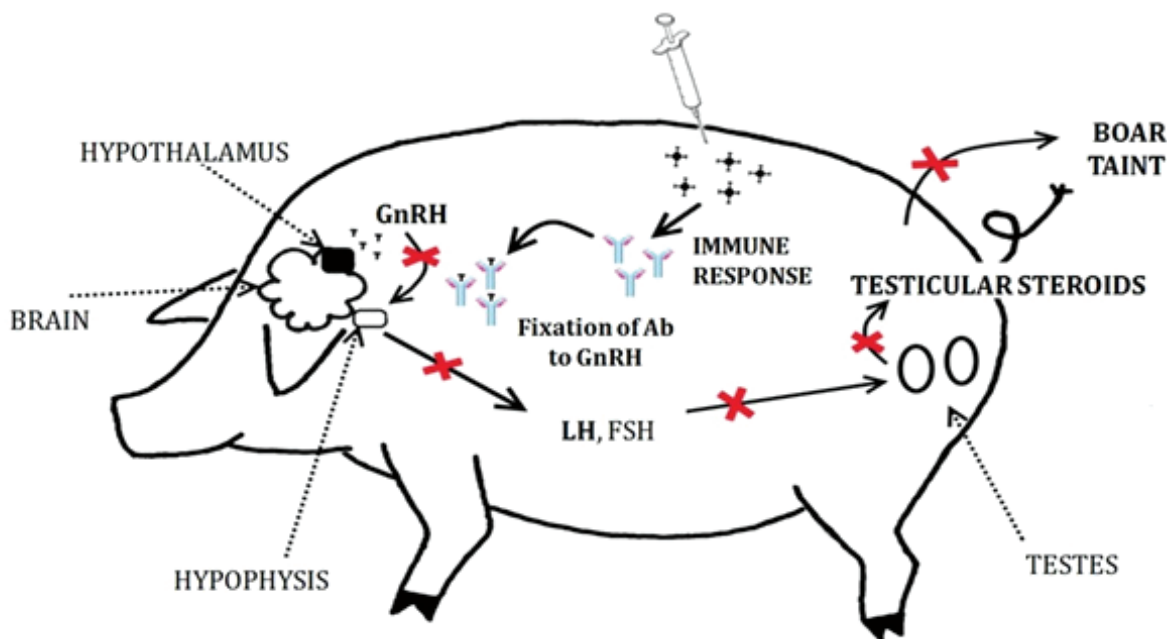


Fig. 2 Physiological response to immunocastration in male pig: after revaccination a large quantity of antibodies against GnRH are produced that neutralise GnRH. As GnRH is no longer available, there is no stimulus for follicle-stimulating hormone and luteinizing hormone release from hypophysis and spermatogenesis and production of androgen hormones in testis is on hold, meaning that there is neither semen production nor male behaviour.

of immunocastrated pigs (IC) are similar to those of EM (Škrlep et al., 2010; Millet et al., 2011 and Dunshea et al., 2013) and better production performance (i.e. growth rate, feed intake, feed efficiency and carcass leanness) of IC compared with SC (for review see Millet et al., 2011 and Batorek et al., 2012) is observed. The only currently available commercial vaccine – Improvac® (Zoetis Florham Park, NJ, USA) was introduced on the market in Australia and New Zealand in the nineties, and is now registered for use in more than 50 countries around the world, including the EU (since 2009). Although the vaccine producer recommends that a 4-week interval between V2 and slaughter is respected, recent studies (Lealiifano et al., 2011 and Kubale et al., 2013) indicated that the concentration of androstenedione and skatole in fat tissue drop below the limit of sensory detection already two weeks after V2 in pigs of commercial slaughter weight. Simultaneously with the decrease of steroid hormones and boar taint compounds concentrations, the effect of immunocastration is visible on the reproductive organs through the reduced size of the testes and accessory sex glands (Bonneau, 2010), with the strongest impact on the size of vesicular gland, followed by the size of the bulbourethral gland. Regression of the reproductive tract is consistent with the loss of functional activity as shown by histological changes of testicular tissue (i.e. atrophy of Leydig cells and germinal epithelium, the cessation of spermatogenesis, the reduction of the size of glandular acini and the disappearance of secretory function of accessory sex glands; Fang et al., 2010; Einarsson et al., 2011 and Kubale et al., 2013). Although studies suggest that the effect of immunocastration persisted up

to 22 weeks (Zamaratskaia et al., 2008) it should not be permanent (Claus et al., 2008). However, an irreversible loss of reproductive capacity can occur following the earlier vaccination protocol (Einarsson et al., 2009). Regarding the effectiveness of immunization, it should be noted that some animals do not react to it (so-called “non-responders”) due to poor immunological response or technically improper vaccination (Zeng et al., 2002; Jaros et al., 2005 and Škrlep et al., 2012). The number of non-responders is relatively low (1-3%) and is expected to be similar to the number of cryptorchids obtained after surgical castration. Thus, there is a need of a fast, simple but effective method for boar taint detection on the slaughter line also when rearing IC. Due to the large variability in size of testes among animals (also due to differences in age and weight), the evaluation of the effectiveness of immunocastration on slaughter line based on the size of testes is not reliable and the size of the accessory sex glands should be taken into account as well (Bonneau, 2010 and Čandek-Potokar et al., 2014), which is rather unpractical.

Sex preselection

The only currently available accurate and potentially cost effective approaches for achieving sex preselection is separating X- from Y-chromosome-bearing sperm with high volume sperm sexing technology (sperm sexing kit; Ter Beek, 2007) or on the basis of the difference in electric charge by using modified flow-cytometer/cell sorter system (Johnson et al., 1989). Cell sorting system can produce about 18 million X-sperm per h of 85-90% of purity (Johnson, 2000) whereas semen sorting kit results

in 70% purity, which gives the possibility of breeding exclusively female offspring. However, both methods are technologically under developed and currently unsuitable for commercial use in the pig (Hofmo, 2006), as the boar semen is rather sensitive to manipulation compared to other animal species and consequently relatively large insemination dose is required (3 billion spermatozoa in 80-100 mL of fresh diluted semen; Dominiek et al., 2011). However, using post-cervical or deep intrauterine insemination allows 3 to 20 fold reduction in the number of spermatozoa in the insemination dose compared to the standard transcervical artificial insemination (Vazquez et al., 2008), but special skills are needed for catheter handling and there is a possibility of damaging cervical or uterine tissue (Dominiek et al., 2011). Controlled-release capsules containing 2.5 billion boar spermatozoa per dose were developed to extend the preservation time of spermatozoa and maximize the efficiency of a single artificial insemination (Vigo et al., 2009) in swine. Thus, sperm sexing and insemination with X-sperm have a great potential in pig production provided that the reproductive performance is optimized by the use of accurate and precise insemination technique and that semen sorting results in a larger quantity of high quality semen, that would meet the market requirements.

Genetic selection for low androstenone level

Genetic selection for low androstenone level is another long-term and welfare friendly solution that is still in development, although selection of breeds that have a natural low incidence of boar taint could provide short-term solution (EFSA, 2004). Sexually mature boars that have low fat androstenone but normal plasma testosterone and luteinizing hormone values have been identified almost 30 years ago (Bonneau, 1987). However, single nucleotide polymorphisms associated with boar taint compounds that can be applied in practical breeding to reduce boar taint in intact boars without aversive effect on sexual maturity have been identified only recently (Moe et al., 2009) and require further research before being implemented in practice.

CONCLUSION

If surgical castration is associated with pain and risk to the health of animals, non-castration is associated with social stress and fighting occurring when boars reach puberty, resulting in skin lesions and ultimate carcass damage. It is more profitable to raise EM pigs due to their enhanced feed conversion ratio and greater carcass leanness; however the quality of meat from boars may be deteriorated by its odour or taste, lower intramuscular fat content and reduced tenderness. Thus, raising EM and applying castration when they approach puberty would mean combining benefits of the two alternatives; benefiting from boar performance during most of the growth-finish period, while avoiding the risk of boar taint and aggression among pigs. This becomes possible with the use of late immunocastration. However, according to current indications it seems that for fresh meat consumption rearing of entire males will be practiced, whereas immunocastration remains a good alternative in

the case of fattening to a greater weight and also, although commercially less important for culled boars.

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ALTERNATIVES TO SURGICAL CASTRATION OF PIGS⁺

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SUMMARY

Castration or gonadectomy is a surgical procedure performed on male pigs in which testis and epididymis are physically removed from scrotal sack, without anaesthesia or analgesia, in the first days of life, mainly to get calmer and fatter pigs that do not exhibit boar taint and to prevent sexual behaviour in group housing. Although generally used, this form of castration has been recently criticized as being painful for the piglets and may be considered even unnecessary in modern pig production with the emergence of new, alternative techniques, especially if pigs are raised for meat consumption where raising entire males would be more efficient. Thus, taking into account the welfare as well as the economic aspects it has been decided at the level of the European Union that pig producers will voluntarily stop castrating until 2018. At the same time, intensive research of alternative methods to surgical castration, diagnostic methods for boar taint analysis and harmonization of analytical methods is being supported. This publication presents alternative methods to surgical castration of piglets without anaesthesia or analgesia – their mode of action and the positive and the negative aspects of their application. According to current indications the most promising alternatives are immunocastration, surgical castration with analgesia or anaesthesia, rearing of entire males, genetic selection on the reduced boar taint and semen sorting technique and subsequently rearing of exclusively female offspring. The latter two methods are long-term, sustainable and animal welfare friendly, whereas the remaining three alternatives are suitable for immediate introduction into practice. It seems that for fresh meat consumption rearing of entire males will be practiced, whereas immunocastration remains a good alternative in the case of fattening to a greater weight.

Key words: pig, castration, immunocastration, welfare, entire males

Abbreviations: SC – surgical castrates, EM – entire males, IC – immunocastrated pigs, EU – European Union, GnRH – gonadotropin releasing hormone, V2 – second vaccination for immunocastration

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