Immunological castration of boars temporarily reduces testosterone concentration, testis size and function, without long-term effects on libido and sperm quality

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<u>ABSTRACT</u>

The objective was to determine the effects of immunocastration (Improvest) on reproductive characteristics in boars. Seventy-two boars were used in a randomized design with three treatments: single injection (**SI**) or double injection (**DI**) of Improvest, and intact controls (no Improvest; **CNT**) (n = 24/group). At wk 10, 15, 20, 25, and 40, blood was collected and serum harvested to evaluate testosterone concentrations via RIA. At wk 25, 18 pigs (n = 6/ group) were sacrificed and testes were removed, weighed, and measured. Libido was assessed at 32, 36, 47, 60, and 63+ wk of age (1 to 5; 1 = no libido; 5 = high libido) and semen collected beyond 60 wk of age. Testosterone concentrations were less for DI boars compared to CNT boars and SI boars at 20 and 25 wk of age ($P < 0.001$), but not different at 40 wk of age. All testicular measurements and weight were less for DI boars compared to SI and CNT boars ($P < 0.001$). There was no treatment effect on libido between 32 to 63+ wk of age. Semen volume, gel weight and total number of sperm cells were not different among treatments. Sperm concentration was greater for DI than SI ($P = 0.011$), and tended to be greater for DI compared to CNT ($P = 0.102$). Sperm motility tended to be greater for DI boars compared to CNT boars ($P = 0.066$). The results show that there are no permanent effects of immunocastration on reproductive characteristics in boars.

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Introduction

Concern for animal welfare has significantly increased during the past decade in the US and many other countries of the world. This concern has had a major impact on animal production and has resulted in changes to what used to be many standard operating procedures. The swine industry has been scrutinized in recent years by animal activist groups and has been making changes to better accommodate the concern for animal welfare. Although the use of gestation stalls that limit sows to standing, sitting, and lying, is currently the main topic of animal welfare discussion in the US, surgical castration (actual removal of the testes) is perhaps next on the list and is already being debated in many countries. The European Union has initiated work to eliminate surgical castration without anesthesia and is planning a voluntary banning of surgical castration entirely by the year 2018 (Ben-Dov et al., 2014). However, there are many issues to consider when contemplating the abolishment of surgical castration. The biggest concern, however, is boar taint, which gives pork a poor odor and palatability when cooked. Immunological castration could potential improve animal welfare is by ridding the industry of surgical castration, and yet still, eliminate boar taint.

Immunological castration has an important place in today's research agenda due to its benefits for animal welfare and its ability to eliminate boar taint. Moreover, studies have shown that immunologically castrated pigs have an increased growth rate (Oliver et al., 2003) and improved energy conversion rate compared to surgically castrated pigs (Zeng et al., 2002), which are production benefits for the farmer. When compared to surgical castration, immunological castration is also becoming the choice among consumers, at least in Europe. In Norway, consumer preference studies have shown that consumers are content with surgical castration of boars only when performed with local anesthesia (Fredriksen et al., 2011), and many European Union consumers prefer immunological castration over surgical castration (Allison et al., 2008). The consumer preference studies show that there has been a shift in public acceptance away from surgical castration to new alternatives, such as immunological castration, which emphasizes the need for further research in the area.

Improvest (Zoetis Animal Health, Florham Park, NJ) is a product commonly used for immunological castration of boars. Moreover, there is potential for use of Improvest as a tool in AI boar selection programs. For example, all boars could receive the first injection of Improvest at 9 wk of age and growth to 24 wk of age could be assessed. If a boar is classified as a cull based on growth performance, then a second injection of Improvest could be administered and the boar marketed 4 wk later for non-discounted prices and without fear of boar taint. If boar growth performance is acceptable, then there would be no second injection of Improvest and the animal would be further evaluated for breeding. This system would be contingent on there being no detrimental effects of the first injection of Improvest on semen quality and libido.

Boar Taint

Before surgical castration or immunological castration can be discussed, one needs to address boar taint. Boar taint is best described as the foul smell and taste of cooked boar meat which is caused by compounds that reside in the fat (Squires et al., 1991). The most concerning, is the intramuscular fat/marbling of pork coming from intact boars. Boar taint is often associated with a urine/fecal odor and taste, which has made selling of intact boars very difficult, having a negative effect on farm profitability. To better understand boar taint and its connection to castration, it is best to examine its direct causes.

Causes of Boar Taint

Research focused on boar taint has been ongoing since the late 1960's and the causes and hormones responsible for it have been well documented. Patterson (1968) discovered that the hormone androstenone (5α-androst-16-ene-3-one) in the fat of intact boars weighing at least 90 kg was the primary cause of boar taint. Androstenone begins to be synthesized in the Leydig cells of the pig testis around the time of sexual maturity and is stored primarily in the salivary glands and in fat. A summary of the synthesis of androstenone can be found in Figure 1. In the salivary glands, androstenone is converted into an active sex pheromone, which helps to accelerate the onset of puberty in gilts and stimulate the lordosis response in females in or near estrus (Squires, 1999; Grindflek et al., 2011). The sex pheromone also plays a role in the establishment of social hierarchy amongst boars. Skatole (3-methylindole) has also been found to be a major cause of boar taint. This compound is mainly produced by gut bacteria and is a product of the breakdown of the amino acid tryptophan. Like androstenone, skatole is also stored in adipose tissue (Squires and Schenkel, 2010; Grindflek et al., 2011). Gilts and surgically castrated males metabolize excess concentrations of skatole, removing it from the body. In boars, however, the metabolism of skatole is slowed down by the action of testicular reproductive hormones such as testosterone and estradiol (Zamaratskaia and Squires, 2008). A summary of the metabolic pathways of androstenone and skatole are depicted in Figure 2.

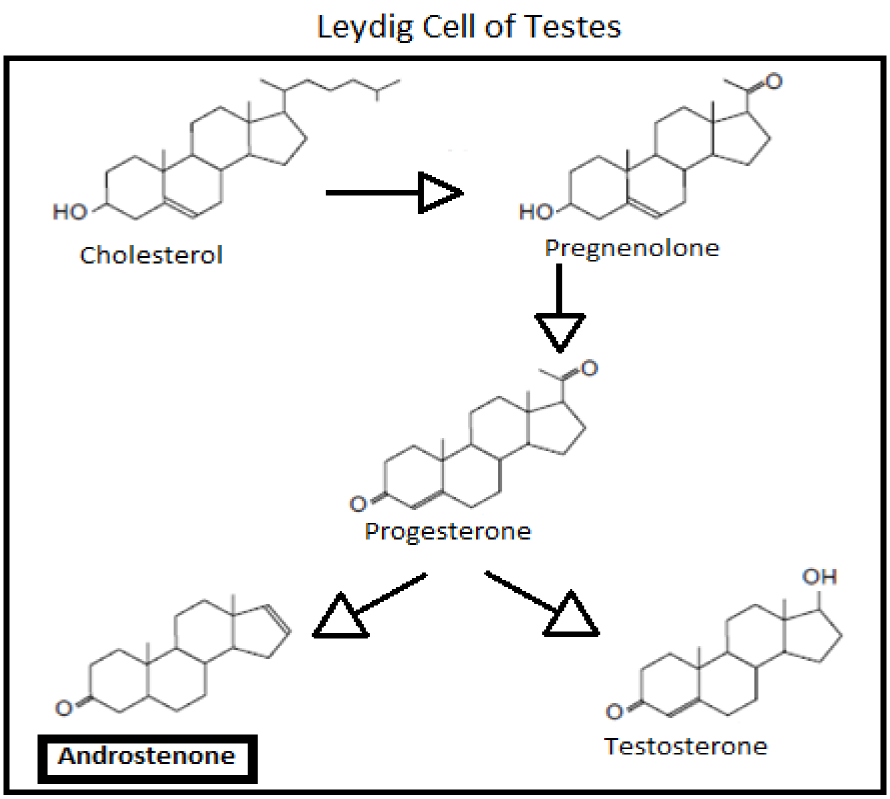

Figure 1. Synthesis of androstenone in the Leydig cells of the testis.

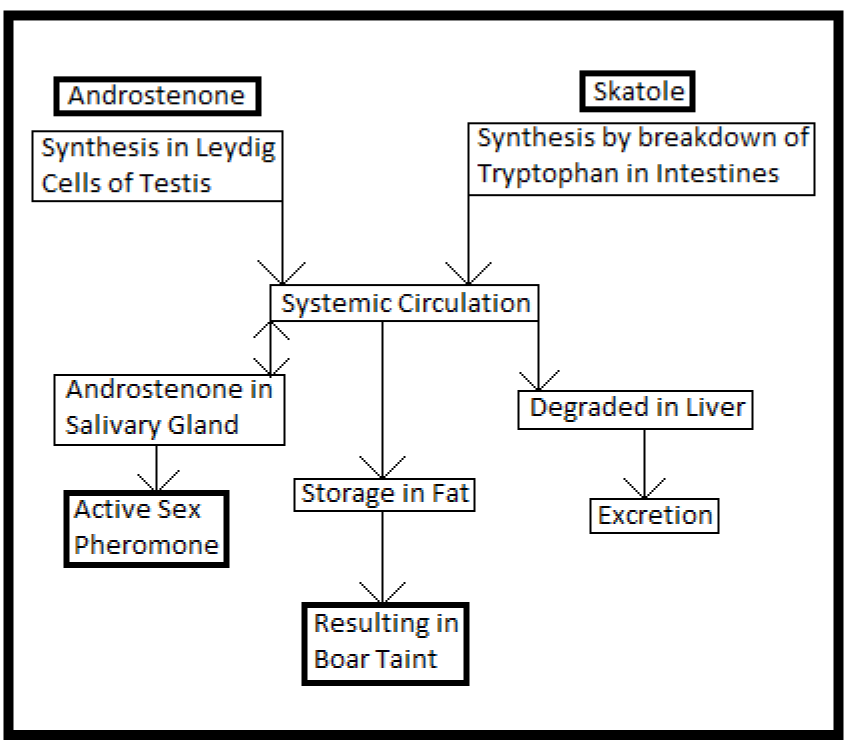

Figure 2. Summary of the metabolic pathways of androstenone and skatole.

Androstenone and skatole concentrations in boars are highly variable. Breed and genetics, and age of onset of puberty in combination with farm management are the main factors contributing to the variation. Xue et al. (1996) found that Duroc and Hampshire boars had greater concentrations of androstenone than Yorkshire and Landrace boars. Fredriksen et al. (2011) also found that Duroc boars showed a greater concentration of androstenone than Landrace boars. Both androstenone and skatole concentrations have been shown to be highly to moderately heritable in all breeds (Squires and Schenkel, 2010; Grindflek et al., 2011). Major genes responsible for boar taint have been located and genetic selection could potentially be used to rid boars of boar taint (Squires, 1999). Genetic selection directly against high levels of androstenone, however, has been correlated with reproductive problems due to decreased levels of other sex steroid hormones (Squires and Schenkel, 2010; Grindflek et al., 2011).

Studies have shown that boar taint compounds can also be affected by management and marketing strategies. Boars fed dry feed, as opposed to wet feed, have been shown to have greater concentrations of both androstenone and skatole (Fredriksen et al., 2011). Skatole can be reabsorbed through the skin from manure in both sexes of pigs, and can cause an increase in boar taint if animals are maintained in dirty conditions (Squires and Schenkel, 2010). Research has shown, that using a management strategy that minimizes mixing of unfamiliar pigs can reduce boar taint. Fredriksen et al. (2011) investigated the use of this system by comparing litters housed together from birth to slaughter to litters that were mixed at 25 kg of BW. The results of this experiment indicated that the mixed litters had greater concentrations of androstenone than the non-mixed litters. This study led to the hypothesis that, like exposing prepubertal gilts to mature boars to stimulate puberty, mixing of unfamiliar boars may also hasten the onset of puberty in males, increasing the concentration of androstenone. Age at puberty is a main source of variation in boar taint in pigs because the concentrations of androstenone produced in the testis have been shown to dramatically increase during the onset of puberty (Fredriksen et al., 2011) and continue to further increase with age (Claus et al., 1971). Intact males can be sold to market with insignificant levels of boar taint if they are sold prior to the onset of puberty.

Detectability and Methods for Detection of Boar Taint

Boar taint detected in meat for human consumption poses an issue for the swine industry because of poor consumer acceptability. The industry accepted sensory thresholds for detecting androstenone is 1.0 μg/g of fat, and for detecting skatole is 0.2 μg/g of fat (Lealiifano et al., 2011). Xue and Dial (1997) reported that approximately 50% of adults cannot detect androstenone in boar meat and 15% can detect it, but are not offended by it. The remaining 35% are hypersensitive to androstenone and find it unacceptable. The researchers also reported that women can detect boar taint more easily than men and that sensory detectability increases with age for men, but decreases with age for women. Also, sensitivity to boar taint increases with continued exposure (Xue and Dial, 1997). However, ninety-nine percent of the general population can detect skatole in boar tainted meat and find it distasteful (Lunde et al., 2010). These human differences in sensibility to boar taint, make marketing intact boars nearly impossible in the swine industry and make detection for boar taint difficult.

One method of detection is via a sensory panel and this technique has been used many times with boar taint preference studies (Squires et al., 1991; Xue and Dial, 1997; Windig et al., 2012). The panel members are trained in detection of boar taint (mainly androstenone) and rate tasted samples based on the presence of boar taint (Xue and Dial, 1997). This method, while generally effective, has some limitations. As mentioned above, human sensitivity to boar taint is variable and can change with continued exposure. More effective and objective detection of boar taint involves measuring the concentrations of boar taint compounds in blood, saliva, and adipose tissue.

Laboratory assays can be performed on serum to determine the concentration of boar taint compounds. Though possible to test for androstenone using RIA, commercial kits are not available, so researchers often test for other steroids that are associated with testicular function, such as testosterone and estradiol. Colorimetric assays can be performed to determine androstenone and skatole concentrations in both adipose tissue (backfat) and the salivary glands (Squires et al., 1991; Babol et al., 1996). Some researchers have utilized fluoroimmunoassays (Fredriksen et al. 2011; Windig et al., 2012) and chromatography (Patterson, 1968; Claus et al., 1971; Dunshea et al., 2001; Chen et al., 2007) to determine concentrations of boar taint compounds in adipose tissue. Most research, involving boar taint concentration determination, has used a combination of the previously mentioned methods.

Intact Boars in the Market

The many advantages of raising intact boars as market hogs will be covered later in this review. There are, however, many challenges associated with marketing of intact boars, the most important of which being the potential for boar taint. With 35% of the general population sensitive and offended by boar taint, it makes marketing intact animals nearly impossible. Some producers market intact boars prior to puberty (< 85 kg BW) to minimize the chances of boar taint in meat (Dunshea, 2010). This is a common practice in Great Britain, Ireland and Spain, where castration of swine is not performed. This marketing strategy comes with certain consequences, typically reduced prices due to a reduction in the size of the retail cuts and a minor risk of boar taint. Using this strategy, the same number of animals are produced, but with lower BW. This results in a reduced quantity of meat produced per farm and typically a reduction in total revenue of 3.9% (Roest et al., 2009). Another strategy is to market intact males at weights typical of market hogs. This strategy typically comes with major discounts due to the increased risk of boar taint. A study by Dunshea et al. (2001) revealed that 73% of pubertal boars had androstenone concentrations of 0.5 µg/g of fat or greater (49% had greater than 1.0 µg/g of fat). Roest et al. (2009) showed that raising intact boars and marketing at typical market hog BW can only be profitable if the percentage of animals with unacceptable boar taint concentrations is less than 2.5%.

Surgical Castration

Surgical castration is currently a standard operating procedure in the swine industries in the US and other countries around the world. Many consumers do not know that surgical castration is performed and many of those that do, do not know the reason for performing the procedure. Surgical castration is performed on pigs for many reasons. Producers often mix sexes of pigs in grazing or commercial housing situations, and the castration of males allows for better reproductive control of the gilts in the herd and prevents unintentional mating. Castrated males typically have higher levels of fat (both backfat and intramuscular fat) than intact males at market weight, which, depending on the market, can either be a benefit or disadvantage of castration (Dunshea, 2010). One of the major reasons to castrate, though, is for worker and pig safety. Compared to barrows, intact male pigs show significantly greater aggressive and sexual behavior at 21 wk of age (Cronin et al., 2003). This increased incidence of aggressive and sexual behavior causes a reduction in time spent eating resulting in less feed consumed (Dunshea, 2010).

The main reason for surgical castration in the swine industry, however, is to prevent the off odor and taste of boar meat, known as boar taint (Rydhmer et al., 2010). As previously mentioned, boar taint is mainly caused by androstenone, which is produced in the Leydig cells of the testicle, and skatole, a product of the microbial breakdown of tryptophan. Removal of the testes prevents androstenone production from those animals. This removal of the testes also causes an increased removal of skatole by the liver due to a decrease in the concentration of testicular steroids, namely testosterone and estradiol, which inhibit skatole metabolism (Zamaratskaia and Squires, 2009). Though surgical castration has many benefits, it also has many negative consequences.

Surgical castration of male piglets is typically performed between 4 and 14 d of age (Harper, 2008). There are many methods for surgical castration, but the following is the most common. The piglets are held by their back legs and two longitudinal incisions are made low on the scrotum using a scalpel. The testes are slowly pulled out from the scrotum, while the thumb is pressed against the pelvis to ensure that the testicular cord breaks. Aseptic methods are used and the wound is sprayed with an antiseptic before the piglet is placed back with the rest of the litter (Harper, 2008). The castration of male pigs is typically a fast procedure that only requires 10 to 15 s to perform by well trained technicians. In the US, producers rarely use local anesthetics or any type of pain mitigation during the castration process. However, producers in other countries around the world have instituted the use of local anesthetics and pain mitigation during the castration procedure, in an effort to address animal welfare concerns. McGlone and Hellman (1988) showed that pain mitigation and local anesthetics effectively reduced the behavioral changes caused by castration at 14 d of age, but not at older ages.

Welfare Concern of Surgical Castration

Surgical castration has been shown to cause piglets pain and stress. Prunier et al. (2005) showed that pigs surgically castrated at 7 or 8 d of age had significantly greater circulating concentrations of cortisol, ACTH, lactate, and epinephrine than sham castrated pigs and non-handled pigs. Increases in cortisol and ACTH concentrations in the circulation are often used as an indicator of stress. Epinephrine is indicative of the fight or flight response and causes muscle glycogen mobilization, potentially causing the release of lactate (Prunier et al., 2005). This study clearly demonstrated that surgical castration causes stress in piglets.

Surgical castration has also been shown to alter behavior (Moya et al., 2008). Castrated piglets spent less time moving around and in the dog sitting posture, and more time huddling, trembling, and scratching compared to sham-castrated pigs. These behavioral changes indicate pain in the animal, and attempts to minimize pain. Castrated piglets have also been shown to spend less time suckling and more time lying down than sham-castrated pigs (McGlone et al., 1993); they also tend to play less and avoid contact with litter mates more than sham-castrated pigs (Moya et al., 2008). Some, but not all, studies have demonstrated that pre-weaning growth rate is decreased by castration. For example, piglets castrated at 1 d of age had significantly decreased growth rates to 20 d of age compared to piglets castrated at 14 d of age (McGlone et al., 1993).

Surgical Castration Legislation around the World

In the Europe Union, there is legislation allowing intact male pigs weighing over 80 kg to enter the food chain, but resulting meat must be specifically labeled (Moya et al., 2008). Norway has set strict regulations on castration and requires it to be performed by a veterinarian who must use an anesthetic (Veissier et al., 2008). In the UK, Ireland, Cyprus, Spain and Portugal, due to public perception and quality assurance programs, surgical castration is not performed by producers, but boars are marketed at body weights of less than 90 kg (Veissier et al., 2008; Tuyttens et al., 2012). In countries like the Netherlands and Switzerland, surgical castration must be performed using local or general anesthetics. In the European Union, many countries have agreed to a voluntary ban of surgical castration by the year 2018 (Ben-Dov et al., 2014). Other countries (Australia, Brazil, New Zealand, and Mexico) have turned to an alternative method, immunological castration, which will be covered in detail later in this review (Estienne and Harper, 2010).

Consumer and Producer Preference

Research has shown negative effects of surgical castration on animal welfare, but what are consumer and producer attitudes toward the use of the procedure in animal agriculture? A consumer preference study in Sweden showed that consumers prefer meat from surgically castrated pigs than intact males, however, they also preferred the procedure of immunological castration over surgical castration (Lagerkvist et al., 2006). This demonstrated consumer preference for alternatives to castration, but also that marketing intact animals was not an option. A Norwegian consumer preference study showed that 60% of consumers did not know that castration was routinely performed, and that consumers preferred immunological castration or surgical castration with anesthesia over surgical castration without anesthesia; this study also showed that animal welfare was the main reason for making that decision (Fredriksen et al., 2011). However, a producer perception study in Flanders, Belgium showed that producers preferred surgical castration without anesthesia over both surgical castration with anesthesia or immunological castration; in that study, the producers felt that castration with anesthesia would add cost and not truly benefit the welfare of the animal and that immunological castration was neither better nor worse than castration without anesthesia (Tuyttens et al., 2012). So, while there is a clear push by consumers in some countries to find and utilize alternatives to surgical castration, producers feel as though surgical castration, as is, is best for swine production and does not need to be changed. These consumer and producer preference studies yield noteworthy results, though questionnaire phrasing may bias the results. For example, producers may have a different opinion of immunological castration once they fully understand what it means and some of its benefits. The consumers may also favor alternatives, even more, and in particular, immunological castration when environmental and behavioral aspects are considered.

Boars vs. Barrows

Compared to barrows, intact boars are typically more feed efficient and at similar market weights are leaner with less backfat (Dunshea, 2010). Feed to gain ratio is less for boars compared to barrows, meaning that they require less feed input for gain in BW (Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002). Because of superior feed conversion efficiency, yield of lean meat, and perhaps ADG, raising intact boars as market hogs could provide economic advantages. Harding (1993) calculated the economic benefits of rearing intact boars compared to barrows and determined that income per hog was greater ($100.11 versus $93.42, respectively) and grow-finish feed costs per hog less ($25.64 versus $27.53). Margin over grow-finish feed cost per hog was $74.47 for boars and $65.90 for barrows (a total advantage of $8.57 per intact boar versus barrow). Xue et al. (1997) estimated that if boars and barrows were sold at the same price, each boar would net $5 to $20 more than each barrow due to improved feed efficiency and lean proportion in carcasses. Enhanced feed conversion efficiency displayed by boars would also result in improved utilization of nitrogen and less excretion of the element into the environment (Xue et al., 1997). Overall, boars also have a greater ADG than barrows

(Dunshea et al., 2001; Turkstra et al., 2002) only if the diet contains an adequate supply of amino acids, particularly lysine, to maximize growth (Xue et al., 1997). However, around the time of puberty, intact boars have a decreased ADG compared to barrows (Zeng et al., 2002). Zeng et al. (2002) showed that surgical castrates had a back fat depth, on average, of 6 and 6.2 mm greater than intact controls on high and low energy diets, respectively. As mentioned, boar taint compounds are stored in the adipose tissue in pigs and have been shown to be significantly greater in intact boars compared to barrows at market weight, with barrows only having trace amounts that can rarely be detected by humans (Xue and Dial, 1997). That being said, intact boars show only trace amounts of boar taint compound in the fat prior to puberty, when there is rapid accumulation of androstenone in the fat (Xue and Dial, 1997).

Boars and barrows not only grow at different rates, compositions and efficiencies, they also display different behavioral characteristics. Intact boars display much more aggressive (27.9 ± 4.66) and sexual bouts (7.2 ± 0.1) each day compared to barrows (9.9 ± 4.66 and 0.6 ± 0.1, respectively; Cronin et al., 2003). Those researchers also showed that, 4 wk prior to slaughter, 74% of surgical castrates were without skin lesions, while only 46% of intact males were without lesions. These aggressive and sexual bouts not only can be damaging to the carcass (causing bruising and lesions), they also cause energy expenditure and a reduction in the amount of time feeding (Cronin et al., 2003). The aggressive behavioral characteristics displayed by intact boars are also a worker safety issue.

Immunological castration

With advances in technology and a growing concern for animal welfare, alternatives to surgical castration are being sought and have been developed. A few of these alternatives include; sexed semen (only using X-chromosome bearing sperm cells thus producing all, or nearly all, gilt litters), immunological castration, and raising intact males (Thun et al., 2006). The advantages and disadvantages of raising intact boars for pork was previously described in this review. Separation of X- and Y-chromosome bearing sperm cells using flow cytometry and insemination of X-chromosome bearing sperm cells could be utilized in the swine industry to produce entire litters of gilts. This procedure, however, is time consuming and, currently, not practical for widespread commercial use (Thun et al., 2006). Immunological castration of swine is the method that will be the focus of the remainder of this review.

To begin, what is immunological castration? Immunological castration, or immunocastration, is the use of an immunization to effectively render the testes nonfunctional through perturbation of the hypothalamic-pituitary-testicular axis. Gonadotropin releasing hormone is produced by the hypothalamus in the brain and is transported to the anterior pituitary through the hypothalamo-hypophyseal portal system. At the pituitary gland, GnRH binds to the gonadotropic cells, which then secrete LH and FSH. These two hormones then travel through systemic circulation to the testes, where they stimulate sperm production and testicular steroid synthesis (Hall, 2011). A visual representation of the hypothalamic-pituitary-testicular axis is shown in Figure 3.

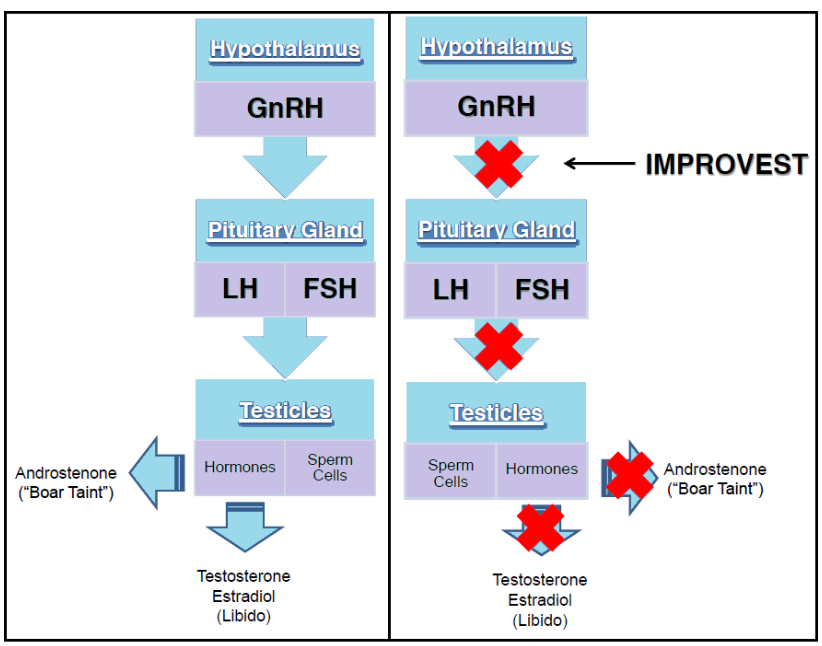

Figure 3. Summary of hypothalamic-pituitary-testicular axis.

Improvest (Improvac) was created by Zoetis Animal Health and is the major immunological castration product available for swine. This product was created with a modified version of GnRH, a GnRH analog, which was conjugated to diphtheria toxoid in an aqueous solution (Zoetis Animal Health, 2013a). This works by eliciting an immune response in the boar, creating antibodies against GnRH, which then bind to endogenous GnRH preventing its entry into the primary portal plexus, or preventing GnRH binding to the gonadotroph receptors on the anterior pituitary gland (Fabrega et al., 2010; Bilskis et al., 2012; Zoetis Animal Health, 2013a).

The first immunization (administered via s.c. injection) is administered as early as 9 wk of age followed by a minimum of 4 wk before the second immunization is given. Once the second immunization is given, there is a 3 wk period during which the animal cannot be marketed. Once that 3 wk period is over, the animal can be marketed as a barrow (even though it still has testes) up until 10 wk post second injection (Zoetis Animal Health, 2013a). The timing of marketing the animal will be explained later. The remainder of this review will explain immunological castration and its effects in detail. After some preliminary information on immunocastration in bulls is presented, swine will become the focus.

Bovine, background and uses

In the beef industry, immunological castration is used for post-pubertal, non-breeding bulls. The product used in bulls is called Bopriva (the only approved GnRH vaccine available for beef cattle), which is also made by Zoetis Animal Health in the same fashion as Improvest, though timing of injections differ (Janett, et al., 2012; Zoetis Animal Health, 2013b). Bopriva is used extensively in South America, as well as New Zealand, Australia, and Central America. The major use of Bopriva is to temporarily decrease circulating concentrations of testosterone in bulls, which last 3 to 4 months, making the bulls easier to manage (Zoetis Animal Health, 2013b). This reduction in testosterone reduces aggressive and sexual behavior of the bulls. The reduction in aggressive and sexual behavior is characterized by a reduction in physical activity, while weight gain is unchanged (Janett et al., 2012). Moreover, the reduction in physical activity decreases homosexual mounting and destruction of pasture (Janett et al., 2012).

Immunological castration is also used in feedlot and pasture settings as an alternative to surgical castration. Surgical castration is routinely performed in many different types of beef operations in order to reduce aggressive behavior, and to improve meat and carcass quality (Amatayakul-Chantler et al., 2012). In South America and tropical settings, surgical castration is performed late in life (around 24 months of age) and animals are harvested at 30 to 36 months of age (Amatayakul-Chantler et al., 2013). This late timing of the castration has many disadvantages including animal welfare concerns, reduced growth performance, and the risk of complications. One potential major complication of surgical castration is the risk of screwworm infections, which in one study occurred at a rate of 8.1%. In contrast, bulls subjected to immunological castration had a screwworm infection rate of 0% (Amatayakul-Chantler et al., 2013). The use of immunological castration effectively prevents testicular production of steroids and a reduction of sperm by 97% (Amatayakul-Chantler et al., 2012). Additionally, there was no reported human exposure due to the use of the safety injector, which was used to give the immunizations (Amatayakul-Chantler et al., 2013).

Bovine, growth performance and meat quality

Immunological castration has been effectively used in cattle as an alternative to surgical castration. This alternative has resulted in cattle that are safer to work with, as well as more growth efficient to market weights. Janett et al. (2012), showed that immunologically castrated bulls grew at a similar rate compared to intact control bulls. Another study in a feedlot setting, showed a tendency for an improved feed efficiency for immunologically castrated bulls that had anabolic steroid implants compared to intact bulls, immunologically castrated bulls, and implanted intact bulls (Amatayakul-Chantler et al., 2012). This same study showed that immunologically castrated bulls had a greater ADG than intact bulls (Amatayakul-Chantler et al., 2012). A pasture-based study showed that immunologically castrated bulls had a greater live weight and ADG than surgically castrated bulls 27 wk after castration or immunocastration (Amatayakul-Chantler et al., 2013).

Carcass and meat quality issues are a concern when raising intact bulls, and a compelling reason for surgical castration. Studies have shown that immunologically castrated bulls have similar carcass characteristics and meat quality compared to surgically castrated bulls when considering pH, fat depth, cooking loss, or tenderness determined by shear force tests (Janett et al., 2012; Amatayakul-Chantler et al., 2013). Immunologically castrated bulls have been shown to have an improved hot carcass weight and dressing percentage compared to surgically castrated bulls (Amatayakul-Chantler et al., 2013). Immunologically castrated bulls have a thicker fat coverage, improved tenderness, and a larger percentage of Choice-graded carcasses compared to intact bulls. However, immunologically castrated bulls seem to have a reduction in the ribeye area compared to intact bulls, although that reduction is not exhibited when the immunological castration is accompanied by an anabolic implant (Amatayakul-Chantler et al., 2012). Overall, immunological castrates have a carcass and meat quality similar to surgical castrates and superior to intact bulls, yet grow as efficient and rapid as intact bulls.

Swine, background and uses

In the US swine industry, surgical castration is routinely performed and the procedure is beginning to be scrutinized by animal welfare activists. Castration is normally performed early in life, between 4 to 14 d of age (Harper, 2008), and, as previously mentioned, undeniably causes pain. Surgical castration not only causes pain, but also compromises growth and performance. Barrows grow slower with more fat deposition and are less feed efficient than intact boars. This decrease in efficiency is caused by the removal of the testes and, more specifically, the removal of the sex steroid hormones. The reduction in growth performance and feed conversion efficiency are major consequences of castration, however, there are also many benefits to the procedure, such as, decreased aggression, removal of boar taint and improved carcass quality. The ideal male market hog, would possess all of these characteristics, including the growth performance of the intact male.

Studies have shown that antibody titers against GnRH are quickly produced after the second injection of Improvest and high levels of anti-GnRH antibodies are maintained for weeks, with no observable injection site reactions (Falvo et al., 1986; Dunshea et al., 2001). Not only is immunocastration effective, consumers seem to favor immunocastration over surgical castration and raising/consuming meat from intact animals. A Swiss consumer preference study revealed that 77% of Swiss consumers preferred immunocastration over surgical castration and 60% said that they would only eat pork from immunocastrated animals, none from surgically castrated animals (Allison et al., 2008). To better grasp immunological castration, the effects on all aspects of swine production need to be assessed.

Swine, altered animal behavior

One negative consequence of raising intact animals, is altered behavior that causes a loss of performance. Immunological castration eliminates negative behaviors, such as fighting and riding, which are generally thought to be caused by sex steroid hormones like testosterone. As will be further covered later in this review, testosterone and other sex steroid hormones produced in the testes are effectively eliminated via immunological castration. The decrease in negative behaviors occur shortly after the second immunization with Improvest and behavior remains unchanged through marketing (Rydhmer et al., 2010).

Focal and scan sample studies have shown that immunologically castrated boars had a significant reduction in the observed aggressive behaviors compared with intact boars. Aggressive behaviors were defined as those intended to injure the other animal with head-butts and biting events (Cronin et al., 2003; Zamaratskaia et al., 2008; Fabrega et al., 2010; Rydhmer et al., 2010). Zamaratskaia et al. (2008) showed that immunological castrates also perform less “manipulating” behaviors compared to intact boars, with those behaviors defined as nibbling or nudging the other animal in a non-aggressive fashion. Intact boars have been observed performing more mounting/sexual behaviors than immunologically castrated boars, along with less nonviolent social behaviors (Cronin et al., 2003; Zamaratskaia et al., 2008; Fabrega et al., 2010; Rydhmer et al., 2010).

The behavioral changes caused by immunocastration have led to a reduction in the amount of observed skin lesions relative to intact boars (Fabrega et al., 2010; Rydhmer et al., 2010). The behavioral changes not only involve pig-pig interactions, but also pig feeding behaviors. Intact boars were observed spending less time at the feeders, than immunologically castrated males and surgically castrated males 3 wk post second immunization (Cronin et al., 2003). While the changes in behavior are a benefit to pig and worker safety, immunological castration can also change the growth rates of boars.

Swine, growth and performance

Compared to intact boars, immunologically castrated pigs have been shown to have an increased amount of time spent consuming feed and improved growth. Average daily gain in immunocastrates has been well-researched, but results are equivocal. Some researchers have demonstrated that ADG for immunocastrates is greater or tends to be greater than for surgical castrates (Zeng et al., 2002; Jaros et al., 2005; Boler et al., 2012; Morales et al., 2013). In contrast, other studies have shown no difference in ADG between immunocastrates and surgical castrates (Falvo et al., 1986; Fabrega et al., 2010). Finally, Morales et al. (2011) reported that immunocastrates had a reduction in ADG compared to surgical castrates. When comparing intact boars to immunocastrates, ADG has been shown to not differ (Bonneau et al., 1994), or for ADG in immunocastrates to be greater than in intact boars (Dunshea et al., 2001; Fabrega et al., 2010).

Turkstra et al. (2002) investigated the pig response to the immunization, categorizing animals as early responders and late responders. Late responders were defined as "animals that had significantly greater concentrations of testosterone than barrows 8 wk after the first immunization" and early responders were defined as "having low concentrations of testosterone (at similar concentrations to barrows) 8 wk after the first immunization." This study showed that the early responder immunocastrates grew similar to surgical castrates, while the late responder immunocastrates grew more like intact boars. Although late responding immunocastrates grew similarly to intact boars, intact boars actually had a greater ADG and a better feed conversion efficiency. The different responses in ADG among studies could be a reflection of the rapidity in which animals responded to immunization or differences in breed of boars, sample sizes and/or diet (Zeng et al., 2002).

Effects of immunocastration on feed conversion efficiency are more consistent. Most studies have shown that immunological castrates are more feed efficient than surgical castrates (Dunshea et al., 2001; Fabrega et al., 2010; Morales et al., 2011; Morales et al., 2013). Intact males have a superior feed efficiency compared to surgical castrates (Bonneau et al., 1994; Zeng et al., 2002). Intact males and immunocastrates generally do not differ in feed efficiency (Bonneau et al., 1994; Fabrega et al., 2010). Zeng et al. (2002) demonstrated that on a high energy diet, intact boars had an improved feed efficiency compared to immunocastrates, but feed efficiency was not different when animals were fed a low energy diet

Swine, effects on boar taint

The major causes of boar taint are androstenone (produced in testes) and skatole (produced by gut micro biota). Research has shown that immunological castration reduces the level of androstenone to low or undetectable levels, whereas about 50% of intact boars have high levels of androstenone (Falvo et al., 1986; Bonneau et al., 1994; Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002; Zamaratskaia et al., 2008; Lealiifano et al., 2011). Immunological and surgical castrates display similar concentrations of androstenone in blood sampled a few weeks after the second immunization (Dunshea et al., 2001; Jaros et al. 2005). The timing of this reduction of boar taint is important for the marketing of these animals. The market window for immunological castrates is 3 to 10 wk after the second immunization. The reason for this is to guarantee that the animals being marketed have no risk for boar taint (Zoetis Animal Health, 2013a). Skatole levels are generally much lower than androstenone, and only about 11% of intact boars have elevated levels of skatole (Dunshea et al., 2001). Skatole is also greatly reduced/undetectable in only a few weeks after the second immunization (Bonneau et al., 1994 Dunshea et al., 2001; Zamaratksaia et al., 2008; Lealiifano et al., 2011). Immunological castration effectively decreases or eliminates boar taint in meat. However, it is important to determine effects of the procedure on carcass quality.

Swine, carcass and meat characteristics

Carcass characteristics and meat quality of intact boars, barrows, and immunological castrates differ. In general, intact boars are leaner, with less backfat and intramuscular fat than surgical castrates (Bonneau et al., 1994; Fabrega et al., 2010). Immunological castrates are generally leaner with less backfat than surgical castrates, but are not as lean as intact boars (Dunshea et al., 2001; Jaros et al., 2005; Morales et al., 2011; Boler et al., 2012; Morales et al., 2013). Lealiifano et al. (2011) performed a study that investigated fat deposition in immunological castrates assessed during the period of time between the second immunization and slaughter. This study revealed that backfat depth was minimized when the time between second immunization and slaughter was decreased. Immunological castrates have been shown to have a reduced yield of meat from the carcass compared to surgical castrates and gilts, and typically have a reduced dressing percentage (Zeng et al., 2002; Morales et al., 2011; Boler et al., 2012; Morales et al., 2013). One study showed that ham and loin weights were greater for gilts than for immunocastrates and surgical castrates (Morales et al., 2013). With that said, hot carcass weight was not different between immunologically castrated and surgical castrated males (Falvo et al., 1986; Boler et al., 2012).

Meat quality for immunologically castrated pigs did not differ from intact males nor surgical castrates in regards to pH, shear force, objective or subjective color scores, or drip loss (Bonneau et al., 1994; Boler et al., 2012). Boler et al. (2012) looked at quality of bacon, and showed that immunocastrates had thinner, narrower bellies than surgical castrates. Falvo et al. (1986) showed that there was no difference in loin eye area for immunological castrates when compared to surgical castrates. With carcass characteristics for immunological castrates well researched, meat quality is one area that seems to need more attention.

Swine, testicular function

The final section of this review will examine the effects on the testes caused by immunological castration. Testosterone is one of the major steroid hormones produced by the testes and plays a role in both reproduction and growth. In boars receiving a two injection series of anti-GnRH, testosterone concentrations in serum increase after the first immunization, but were then reduced after the second immunization compared to intact boars (Falvo et al., 1986; Bonneau et al., 1994; Zeng et al., 2002; Zamaratskaia et al., 2008; Bilskis et al., 2012). Zamaratskaia et al. (2008) showed that serum testosterone concentrations remained significantly less in immunocastrates than intact boars through 22 wk post second injection, which is the point at which that particular experiment ended. Testosterone concentrations were positively correlated with libido and volume of ejaculate, and negatively correlated with abnormal spermatozoa (Bilskis et al., 2012).

Testes and accessory sex gland weights were significantly reduced in immunologically castrated boars when compared to intact boars (Falvo et al., 1986; Bonneau et al., 1994; Turkstra et al., 2002; Jaros et al., 2005; Zamaratskaia et al., 2008; Einarsson et al., 2009; Lealiifano et al., 2011). Lealiifano et al. (2011) showed that the testes of immunologically castrated boars were lighter and had less redness than intact boars, which they showed was related to a decrease in testicular hemoglobin and mitochondrial activity.   Immunological castrates have been shown to be sexually inactive through market weight and in one study up to 22 wk post second injection

(Zamaratskaia et al., 2008; Einarsson et al., 2009). Einarsson et al. (2009) evaluated sperm harvested from the caudal epididymis in immunological castrates and control boars. Intact boars had a lower percentage of sperm cells with proximal droplets and abnormal heads, but a greater percentage of sperm cells with distal droplets compared with immunological castrates 4 wk post second immunization. However, at 16 and 22 wk post second immunization, immunological castrates had a larger percentage of sperm cells with proximal and distal droplets, acrosomal defects and abnormalities, and abnormal heads than did sperm cells from intact boars. Though the previous study was able to show the differences in sperm quality, it did not evaluate libido.

Bilskis et al. (2012) investigated the efficacy of immunological castration on testosterone concentration, libido, and sperm quality in culled AI boars using three immunizations. This study used 9 cull AI boars. The trial was split into four time periods: control period before immunization, from 1st immunization to the 2nd, 2nd to the 3rd immunization, and 3rd immunization to 4 wk post 3rd immunization. All immunizations were given at 4 wk intervals and each boar was its own control, and thus treatment and time were confounded. The researchers did show, however, that testosterone concentrations and libido increased after the 1st immunization, followed by a decrease after the 2nd and 3rd immunizations. Volume of ejaculate decreased after the 2nd and 3rd immunizations, while abnormal sperm increased. Though volume of ejaculate decreased after the 2nd and 3rd immunizations, sperm concentration and motility remained the same. This study did not use unvaccinated control boars and, therefore, further investigation is needed.

The Bilskis et al. (2012) study did have some potential implications for boar stud operations. Cull AI boars may potentially be given a few immunizations and then sent to market. Similarly, boar stud personnel could use immunological castration as a tool in boar selection protocols. Boar studs have to select boars either at an early age before growth performance is adequately assessed, or select when boars are older. The non-selected animals are sent to market as intact boars, resulting in price deductions. In theory, all boars could receive the first injection of Improvest at 9 wk of age and growth to 24 wk of age assessed. If a boar is classified as a cull based on growth performance, then a second injection of Improvest could be administered and the boar marketed 4 wk later for non-discounted prices and without fear of boar taint. If boar growth performance is acceptable, then there would be no second injection of Improvest and the animal would be further evaluated for breeding. This system would be contingent on there being no detrimental effects of the first Improvest injection on semen quality and libido.

Conclusions

Though there have been many research studies investigating the efficacy and general effects of immunological castration, there is still much work to be peformed. In the areas of growth and performance, there need to be more studies that investigate ADG, especially comparing immunocastrates to intact boars. The efficacy of a two immunization series has been documented, however, there have been few studies that have investigated potential effects of a single immunization on growth performance, boar taint, and testicular function. Thus far, research efforts have not been devoted to investigating if the effects of immunocastration on testicular function are permanent, and, if not, comparing semen quality from immunological castrates with that of intact boars. This strategy of immunization could be utilized by seedstock producers as a tool during boar selection, and, if there are no detrimental long term effects of a single immunization on semen quality, could be a major economic benefit.

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Chapter 2

Immunological castration of boars temporarily reduces testosterone concentration, testis size and function, without long-term effects on libido and sperm quality.

Introduction

Castration is routinely performed on swine farms when male piglets are between 4 and 14 d of age (Harper, 2008). The main reason for castrating boars destined for market is to prevent the tainting of the pork (Rydhmer et al., 2010). Boar taint is the off odor and flavor of heated boar meat (Squires et al., 1991; Xue and Dial, 1997). Approximately 35 percent of consumers are hypersensitive towards boar taint and describe it as offensive (Xue and Dial, 1997). Compounds responsible for boar taint are the hormone androstenone, which is produced in the Leydig cells of the testes, and skatole, a product of microbial breakdown of tryptophan in the gut (Patterson, 1968; Squires and Schenkel, 2010; Grindflek et al., 2011).

Surgical castration is an emerging animal welfare concern because it has been shown to cause pain and stress in piglets and the procedure is typically performed without anesthesia or analgesics (McGlone et al., 1993; Prunier et al., 2005; Moya et al., 2008). Prunier et al. (2005) revealed that surgically castrated piglets had greater circulating concentrations of cortisol, ACTH, and epinephrine (all indicative of stress) than sham-castrated males. McGlone et al. (1993) and Moya et al. (2008) investigated behavioral responses and showed that surgically castrated piglets spent less time moving around and suckling, and more time huddling, trembling, and isolating themselves from litter mates than sham-castrated piglets. Surgical castration is under scrutiny because it undeniably causes pain and stress in piglets and alternatives to surgical castration are being researched.

Immunological castration renders the testes nonfunctional (Fabrega et al., 2010; Bilskis et al., 2012; Zoetis Animal Health, 2013). It does so by eliciting an immune response and antibody production against GnRH, which is secreted by the hypothalamus (Fabrega et al., 2010; Hall, 2011; Bilskis et al., 2012; Zoetis Animal Health, 2013). Normally, GnRH travels through the hypothalamic-hypophyseal portal system, binding to gonadotropes on the anterior pituitary gland and stimulating LH and FSH release (Hall, 2011). Luteinizing hormone and FSH then travel to the testes through the systemic circulation to stimulate steroid hormone production and spermatogenesis (Hall, 2011). Antibodies produced against GnRH, however, prevent its passage and binding to the gonadotropes, in turn preventing LH and FSH secretion (Fabrega et al., 2010; Bilskis et al., 2012; Zoetis Animal Health, 2013).

Zoetis Animal Health (Florham Park, NJ) has created an immunological castration product, Improvest, which was made with a modified version of GnRH conjugated to diphtheria toxoid (Zoetis Animal Health, 2013). The immunization is a two injection series with the primary dose given around 9 to 12 wk of age and the second immunization given a minimum of 4 wk after primary immunization. The boar may be marketed 3 to 10 wk after the second immunization, and is considered a barrow (Zoetis Animal Health, 2013). Immunological castration has been shown to eliminate boar taint, along with reducing circulating concentrations of testosterone and estradiol (Falvo et al., 1986; Bonneau et al., 1994; Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002; Jaros et al., 2005; Zamaratskaia et al., 2008; Lealiifano et al., 2011).

Use of immunological castration could be advantageous for farmers producing breeder boars. Currently, growth to 24 wk of age is assessed and boars displaying unacceptable performance are culled. These animals are sold at a severely discounted price because of the potential for boar taint. We hypothesize that immunological castration could be used in the following strategy: All boars receive Improvest at 9 wk of age and growth to 24 wk of age is assessed. If a boar is classified as a cull based on growth performance, then a second injection of Improvest is administered and the boar marketed 4 wk later for non-discounted prices, and without fear of boar taint. If boar growth performance is acceptable then there would be no second injection of Improvest and the animal would be further evaluated for breeding. This system is obviously contingent on there being no detrimental effects of the first Improvest injection on semen quality and libido. The objective of our study was to investigate the long term effects of single or double immunization with Improvest on BW, semen quality, reproductive hormone concentrations, and libido score.

Materials and Methods

Animals and Protocol

Animals for this study were born and reared at the Virginia Tech-Tidewater Agricultural Research and Extension Center (**TAREC**) in Suffolk, VA and all animal procedures were carried out in accordance with the guidelines and prior approval of the Virginia Tech Institutional Animal Care and Use Committee. This study utilized a total of 72 Landrace x Yorkshire boars in a randomized design. The boars were divided into two replicates (n = 45 in group 1 and n = 27 in group 2), and within each group, boars were randomly divided into one of three treatment groups: A single immunization (**SI**), a double immunization (**DI**), and an intact control group (**CNT**). Immunizations (Improvest; Zoetis Animal Health, Florham Park, NJ) were given by a trained Zoetis representative using the safety injector in accordance with Zoetis Animal Health guidelines. Immunizations were given s.c. immediately caudal to the ear at 10 wk of age (SI and DI) and at 15 wk of age (DI only). Boars were housed in pens of nine boars each (n = 3 per treatment group per pen) and were fed on an ad libitum basis commercial grower and finisher diets that met the recommendations for the various nutrients. All boars were weighed at birth (1 d old), weaning (3 wk of age), and at 10, 15, 20 and 25 wk of age, and ADG was calculated.

Blood collection and hormone analyses

Blood was harvested from all boars at 10, 15, 20, and 25 wk of age via jugular venipuncture using a 20 ga, 25.4 mm vacutainer needle (Kendall Monoject, Covidien, Mansfield, MA). Blood was also sampled from a subset of boars (n = 10 per treatment group) at 40 wk of age. Collected blood was allowed to clot for 24 hr at 4ºC and was then centrifuged at 4ºC and 1, 800 x g for 30 min. Serum was removed by transfer pipettes, placed into polypropylene tubes, and stored at -20 ºC until hormone assays were performed. Testosterone and estradiol concentration in serum were determined using commercially available RIA kits (Siemens Medical Solutions, Inc., Malvern, PA). Both assays were validated for porcine serum (Estienne et al., 2004) and were performed using the protocols provided with the kits. The intra-assay CV was 5.53% for the testosterone assay and 8.16% for the estradiol assay. Assay sensitivity was 0.04 ng/mL for the testosterone assay and 0.01 ng/mL for the estradiol assay.

Testicular measurements

Testicular measurements were performed after 18 boars (n = 6 per treatment) were killed at 25 wk of age (penetrating captive bolt followed by exsanguination). The testes were dissected out of the scrotum and the epididymides were removed. Each testicle was weighed (Acculab, Minitube of America, Inc, Verona, WI). Circumference was measured latitudinally around the midpoint of the testes using a cloth measuring tape. Testicular length and width were measured using a caliper.

Tissue sampling

Tissue samples were also collected from the 18 animals (n = 6 per treatment) killed at 25 wk of age. Backfat samples (5x5x2 cm sections) were collected at the base of the neck from each animal. The backfat samples were placed in whirl pak bags (Nasco, Modesto, CA) and flash frozen in liquid nitrogen. The samples were shipped to Iowa State University for boar taint aroma evaluation performed by a trained sensory panel. Fat samples were cut in half, placed in Styrofoam cups, and were heated for 20 sec on high power in a conventional microwave oven. Samples were then distributed to the sensory panel and boar taint aroma was scored on a scale of 0 to 15, with a score of 0 representing no boar taint aroma detected and 15 representing strong boar taint aroma detected. Panel members were blind to animal identification and treatment group.

Semen collection and evaluation

Training of boars for semen collection began around 60 wk of age. Boars were trained to mount an artificial sow and semen was collected using the gloved hand technique. During the training process, boars were evaluated and scored for libido on a five point scale: 1-boars showed no interest in artificial sow; 2- slight interest in artificial sow but did not attempt to mount; 3 – mounted the artificial sow but did not display an erection; 4 – mounted artificial sow and displayed an erection, but did not allow semen collection; and 5 – mounted the artificial sow and allowed semen collection (Kozink et al., 2002). Boars were collected four times (2 collections per wk per boar) before semen was evaluated, followed by four more collections (2 collections per wk per boar) during which semen was evaluated.

During collection, semen was filtered (US BAG, Minitube of America, Inc.) to remove the gel. Gel-free volume and gel weight were determined gravimetrically using a top-loading balance (Acculab; Minitube of America, Inc.). Sperm concentration was determined using a Spermacue photometer (Minitube of America, Inc.). Percentage of motile sperm was subjectively determined by examining spermatozoa (100x) using an Olympus phase contrast trinocular microscope with heated stage (37ºC) (Olympus America, Inc., Melville, NY).

Statistical Analysis

Statistical analysis was performed using the PROC MIX function in SAS 9.3 (SAS Institute, Inc., Cary, NC). Repeated measures were performed on BW, ADG, testosterone and estradiol, libido and semencharacteristics. Repeated measures on ADG were performed on ADG for the 5 wk time intervals during the trial period (10 to 15 wk, 15 to 20 wk, and 20 to 25 wk). Compound symmetry, unstructured, autoregressive, and heterogeneous autoregressive covariant structure were tested to minimize akaike information criterion (**AIC**). For BW, ADG, and hormone concentrations in serum, the models included week, pen, and treatment as possible sources of variation. The BW at 10 wk of age was used as a covariate for BW at 15, 20, and 25 wk of age and for ADG from 10 to 25 wk of age. The models for testicle measurements and boar taint aroma analysis included pen and treatment as possible sources of variation. Body weight, ADG, and hormone concentrations in serum statistical analyses consisted of 24 experimental units per treatment. Testicular measurements and boar aroma score statistical analyses consisted of 6 experimental units per treatment. Boar taint (present or not present) was analyzed using Chi Square analysis. For libido and semen data, the models included treatment and collection number as possible sources of variation. Libido score data consisted of 10 experimental units per treatment and boar semen data consisted of 3 experimental units per treatment with 12 observational units per treatment. Where appropriate, Tukey-Kramer mean separation was used to compare LS mean differences. Differences were considered significant with a *P* value less than 0.05 and were considered tendencies if the *P* value was between 0.12 and 0.05.

Results and Discussion

Body Weight and ADG

Body weight at 10 wk of age did not differ among treatment groups (*P* = 0.889). Body weights were affected by week (*P* < 0.001), tended to be affected by treatment *P* = 0.077), and was not affected by treatment x week (*P* = 0.522). Body weight between 15 and 25 wk of age tended to be greater for DI boars compared to SI boars (*P* = 0.077), but was not different for DI compared to SI boars and SI compared to CNT boars (*P* = 0.547 and *P* = 0.410, respectively). Body weight data is summarized in Table 1. The present study showed no differences between treatment groups in BW from birth through the initiation of the trial period (10 wk of age). After immunization and through market, the DI boars tended to have a greater BW than the intact males. Other studies have shown that DI boars have a significantly greater BW at market age (25 wk of age; Dunshea et al., 2001; Rikard-Bell et al., 2009; and Fabrega et al., 2010). However, other studies showed no difference in BW at harvest between intact boars and immunological castrates (Bonneau et al., 1994; Wicks et al., 2013). The study by Wicks et al. (2013) had only 6 intact control boars compared to 20 immunologically castrated boars and is likely to be the reason no difference was seen in BW. In the Bonneau et al. (1994) study, boars were immunized based on BW, not on age. Lealiifano et al. (2011) have been the only researchers (aside from the present study) to compare BW in boars after single or double injections of Improvest. Both studies showed no difference in BW at slaughter between SI and DI treatments.

Average daily gain data is summarized in Table 1. Average daily gain of boars was determined for the period prior to treatment (birth to 10 wk of age) and for for each five wk interval in the trial (10 to 15 wk of age, 15 to 20 wk of age, and 20 to 25 wk of age). Repeated measures were used to analyze ADG for the period from 10 wk of age until the end of the experiment. From birth to the start of the trial, ADG did not differ among treatment groups (*P* = 0.921). Avergae daily gain was affected by treatment (*P* = 0.032) and week (*P* < 0.001), but not treatment x week (*P* = 0.944). Average daily gain was greater for DI boars compared to CNT boars for the trial period (*P* = 0.032) and was not different between DI and SI boars and SI and CNT boars (*P* = 0.606 and *P* = 0.208). The results of the present study agree with results comparing DI and CNT presented in other studies in that prior to immunization, there was no difference in ADG (Turkstra et al., 2002; Zeng et al., 2002). Lealiifano et al. (2011) have been the only other researchers to investigate ADG after a single immunization and reported no difference for SI and DI boars. In the present study, DI boars grew faster than CNT boars, which has been shown in previous research (Zeng et al., 2002; Oliver et al., 2003; Fabrega et al., 2010). These results are contradicted by Turkstra et al. (2002) who reported that intact boars had a greater ADG than immunological castrates. In that study, however, boars were restricted fed from the start of the study, which is likely the reason for a greater ADG for CNT boars than DI boars. Turkstra et al. (2002) also assessed feed conversion efficiency and reported that intact boars had a greater feed efficiency than immunological castrates. Therefore, by restricting feed, boars grew faster than immunocastrates.

<u>Estradiol and Testosterone Analyses</u>

Serum samples were harvested and hormone RIA performed for blood samples collected at 10, 15, 20 and 25 wk of age. Estradiol concentration data is summarized in Table 2. There was an effect of treatment x time (*P* < 0.001) for estradiol. At 10 and 15 wk of age, estradiol concentrations did not differ among treatment. At 20 wk of age, DI boars had a reduced concentration of estradiol compared to the SI and CNT boars (*P* < 0.001) and SI and CNT boars were not different. At 25 wk of age, DI boars had a reduced estradiol concentration in serum compared to the SI (*P* = 0.001) and CNT boars (*P* = 0.002) and SI and CNT boars did not differ. Estradiol has been used as an indicator of testicular function in boars (Zamaratskaia and Squires, 2009; Lugar and Estienne, 2013). The results in the present study are in concurrence with other studies investigating the effects of immunological castration on estradiol production (Zamaratskaia et al, 2008; Kubale et al., 2013). Kubale et al. (2013) showed that estradiol concentrations in intact boars increased significantly around 19 wk of age and revealed the second immunization of boars around 19 wk of age halted this increase and concentrations decreased thereafter.

Testosterone concentrations in serum were analyzed for blood sampled at 10, 15, 20, and 25 wk of age. For testosterone, there were effects of treatment, week and treatment x week ($P < 0.001$). A subset of boars (n = 10 per treatment) were also sampled at 40 wk of age. Testosterone concentration data is summarized in Table 2. At 10 and 15 wk of age, testosterone concentrations did not differ among treatment. At 20 wk of age, DI boars had lesser concentrations of testosterone compared to CNT and SI boars ($P < 0.001$) and concentrations were not different between SI and CNT boars. At 25 wk of age, DI boars had a lesser testosterone concentration compared to SI and CNT boars ($P < 0.001$) and concentrations were not different between SI and CNT boars. At 40 wk of age, there was no difference in testosterone concentration among treatment groups (*P* = 0.473).

Testosterone concentration was used as an indicator of testicular function in boars for the present study. Boars given two immunizations of Improvest had significantly reduced concentrations of testosterone at five wk post second injection compared to intact boars, which agrees with results from previous studies (Falvo et al., 1986; Bonneau et al., 1994; Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002; Zamaratskaia et al., 2008; Kubale et al., 2013;

Wicks et al., 2013). Studies have shown that, in general, testosterone concentration is significantly reduced around 2 to 3 wk after the second immunization (Falvo et al., 1986; Dunshea et al., 2001; Zeng et al., 2002; Wicks et al., 2013). Lealiifano et al. (2011) showed that boars receiving only one immunization had significantly greater concentrations of testosterone than boars receiving two immunizations. Moreover, this study showed that in blood collected at slaughter, boars given the second immunization 6 wk before slaughter had significantly greater concentrations of testosterone than boars given the second immunization 2 wk before slaughter. These results indicate that the effects of immunization on testosterone decrease with time post second immunization. The present study also showed greater concentrations of testosterone at 25 wk of age compared to 20 wk of age in DI boars (1.178 ng/mL and 0.065 ng/mL, respectively).

The present study is the first to measure testosterone concentrations at 40 wk of age in DI boars and showed that the testosterone concentration was not different from intact or single injection boars. The study by Lealiifano et al. (2011) indicated that the effects of immunization decline with time, which is clearly shown in the results of the present study. Serum testosterone concentrations at 40 wk of age (25 wk post second injection) indicate that testicular steroid production resumed to levels seen in intact boars.

Testicular Measurements

At 25 wk of age, 18 boars were sacrificed and the testes of each boar were removed, weighed, and measured (Table3). The left and right testicle weights did not differ between SI and CNT ($P = 0.982$ and $P = 0.971$, respectively), and were greater for SI and CNT boars compared to DI boars ($P < 0.001$). The left and right testicle circumference did not differ between SI and CNT boars ($P = 0.994$ and $P = 0.999$, respectively) and was greater for SI and CNT boars compared to DI boars ($P < 0.001$). Left and right testicle lengths did not differ between SI and

CNT boars (*P* = 0.932 and *P* = 0.853, respectively) and were significantly greater for SI and CNT boars compared to DI boars (*P* < 0.001). Left and right testicular width did not differ between SI and CNT boars (*P* = 0.982 and *P* = 0.886, respectively) and was greater for SI and CNT boars compared to DI boars (*P* < 0.001). Single immunizations had no effect on testicular weight and measurements compared to intact controls, and double injection caused significant reductions in testicular weight and measurements. The results of the present study agree with results from other studies (Falvo et al., 1986; Bonneau et al., 1994; Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002; Oliver et al., 2003; Jaros et al., 2005; Zamaratskaia et al., 2008; Einarsson et al., 2009; Lealiifano et al., 2011; Kubale et al., 2013; Wicks et al., 2013). Lealiifano et al. (2011) also investigated a single immunization of Improvest and showed that testicular length, width, and weight were significantly greater than in boars given the second immunization at least 3 wk before slaughter. This study, however, did not include an intact control group with no immunizations. The present study, is the only that has compared testicular measurements in boars given two immunizations or a single immunization to intact boars. The data from the present study confirms data found in previous studies demonstrating a reduction in growth of the testes from double immunized boars and also confirms that single immunizations have no effects on testicular growth.

<u>Boar Aroma Panel Analysis</u>

Back fat tissue samples collected from 18 boars (n = 6 per treatment) were harvested at 25 wk of age for boar aroma panel analysis by a trained sensory panel. Boar taint was observed in six of the 18 samples sent for evaluation. Three intact boars (50% of CNT) and three single injection boars (50% of SI) had fat samples that had boar taint aroma scores greater than one. Chi square analysis of the data showed that DI boars had significantly less boar taint (no boar taint;

0% of DI) than the SI and CNT boars (*P* = 0.046). The DI boars also had significantly lower boar taint than the average of SI and CNT boars (*P* = 0.034). The results of the present study agree with previous studies that have investigated boar taint levels in double injection boars compared to intact boars (Bonneau et al., 1994; Dunshea et al., 2001; Turkstra et al., 2002; Zeng et al., 2002; Jaros et al., 2005; Zamaratskaia et al., 2008; Kubale et al., 2013). Lealiifano et al. (2011) reported that SI boars had concentrations of androstenone 7 times greater than DI boars.

Libido and Semen Analysis

Libido scores were given during training sessions with the boars at 32, 36, 47, 60 and 63 plus wk of age. Libido score data is summarized in Table5. At 32 wk of age, libido score was not different among treatments, though it was numerically greater for SI and CNT boars compared to DI boars (1.5, 1.5 and 1.0, respectively; *P* = 0.148). Repeated measures were analyzed from 32 to 63 plus wk of age. There was an effect of week (*P* = 0.025) but no effects of treatment (*P* = 0.412), or treatment x week (*P* = 0.713). There was no treatment effect for libido from 32 to 63 plus weeks of age (*P* = 0.412). Libido score increased with time across all treatment groups. This is the first study to analyze libido in immunologically castrated boars. Zamaratskaia et al. (2008) investigated the sexual behavior of boars treated with two injections of Improvest and intact boars comparing them in a 7 min mating test with sows/gilts in estrus. This study showed that most of the intact boars mounted a female in estrus quickly after initiation of the mating test, however none of the immunologically castrated boars mounted. In this study, however, three of the immunological castrates had to be pulled from the test due to fighting with the sow/gilt (in two cases the sow/gilt attacked the male at initiation of the test). The study also showed that at 15 wk post second immunization, two immunological castrates attempted to mount the female, but were unsuccessful. The results of the present study showed no significant difference in libido for DI, SI and CNT boars at 17 wk post second injection (32 wk of age), though DI boars had a numerically lower libido score.

Another study, by Bilskis et al. (2012), investigated the effects of immunological castration on mature, AI boars. Although this study did not contain true controls and was confounded with time, it showed a decrease in libido in the boars after three immunizations. The researchers did not indicate how long the reduction lasted. The present study shows immunological castration does not have a permanent effect on libido score. The present study is the first study to show that libido is no different than intact control boars at 17 wk post second immunization, which indicates that the immunization has a temporary effect on testicular function. Moreover, the present study also confirms that a single immunization of Improvest has no effect on libido in boars.

Semen analysis was performed on a subset of 9 boars (n = 3 per treatment; each boar was evaluated 4 times) after the boars were 60 wk of age. All boars tested were evaluated for semen volume, gel weight, sperm concentration, total sperm, and sperm motility. Semen analysis data is summarized in Table5. Semen volume, gel weight and total sperm did not differ among treatment groups ($P = 0.555$, $P = 0.837$, and $P = 0.409$, respectively). Sperm concentration was greater for DI boars compared to SI boars ($P = 0.011$), tended to be greater for DI boars compared to CNT boars ($P = 0.102$), and did not differ for SI and CNT boars ($P = 0.580$). Sperm motility tended to be greater for DI boars compared to CNT boars ($P = 0.066$), and was not different for DI and SI boars nor SI and CNT boars ($P = 0.536$ and $P = 0.225$, respectively).

The present study is the first study to evaluate semen quality repeatedly on DI and SI boars. The results of the present study indicate that the temporary effect of immunization does not have long-term effects on semen quality. Other studies have shown long-term effects on sperm morphology up to 22 wk post second immunization on DI boars. Wicks et al. (2013) reported that DI boars had decreased amounts of daily sperm production compared to CNT boars 10 wk post second immunization, evaluated from homogenized testicular samples. Einarsson et al. (2009) investigated the short- and long-term effects of immunocastration at 4, 16, and 22 wk post second immunization. In that study, DI boars had a greater incidence of proximal droplets and head abnormalities, but fewer distal droplets than CNT boars at 4 wk post second injection. At 16 and 22 wk post second injection, DI boars also had greater numbers of acrosomal abnormalities and defects compared to CNT boars. Bilskis et al. (2012) investigated the effects of immunological castration on mature, AI boars and reported that DI boars had decreased semen volume, and increased abnormal spermatozoa (proximal droplets, bent tails, and head abnormalities) compared to pre-immunization evaluations. No differences in sperm motility or concentration after immunization were reported. This study did not utilize control boars and thus was confounded with time. The present study showed that any immunological castration effects on semen volume, gel weight, sperm concentration, total sperm and sperm motility are no longer present 45 wk post second immunization. The present study showed no difference in semen volume, gel weight and total sperm for DI boars compared to CNT boars, and sperm concentration and sperm motility tended to be greater for DI boars compared to CNT boars. Single immunized boars had semen volume, gel weight, sperm concentration, total sperm and sperm motility that did not differ from CNT boars. Though these results are promising for potential use of immunological castration in boar stud facilities, semen characteristics were evaluated for a limited number of animals. The present study only had 3 experimental units per treatment for semen evaluation, with 12 observations per treatment. Future studies should continue to investigate the long-term effects of immunological castration on SI and DI boars.

Further research is needed to investigate the length of time from second immunization until the effects of the immunization are no longer seen. Furthermore, future studies should investigate whether the effects of immunization on sperm morphology are temporary, as well.

Conclusions and Implications

The present study has confirmed that a single immunization of Improvest does not affect growth or reproductive performance in boars. However, double immunization with Improvest at 10 and 15 wk of age has short-term effects on testicular growth, sex steroid hormone concentration, and boar taint in boars. The effects of immunization are temporary with libido levels of DI boars no different than CNT boars at 32 wk of age and semen quality returning by 60 wk of age. The present study has shown that SI boars have semen quality characteristics similar to CNT boars. Further research should be conducted to confirm that immunocastration's effect on sperm morphology is also unaffected in SI boars and returns to CNT boar levels for DI boars. The present study indicates that immunological castration could be effectively utilized in boar stud farms without long-term effects on reproductive success in boars. The use of immunological castration in boar studs could increase profitability by eliminating the discounted prices seen when marketing cull boars. The results of the present study indicate that it is possible to give all boars two immunizations of Improvest, make selection based on growth performance at market weight, and then make further evaluations and selection around 32 to 40 wk of age. Though it may be possible to give all boars two immunizations, more research needs to be conducted to evaluate the length of time necessary for reproductive performance to return to normal.

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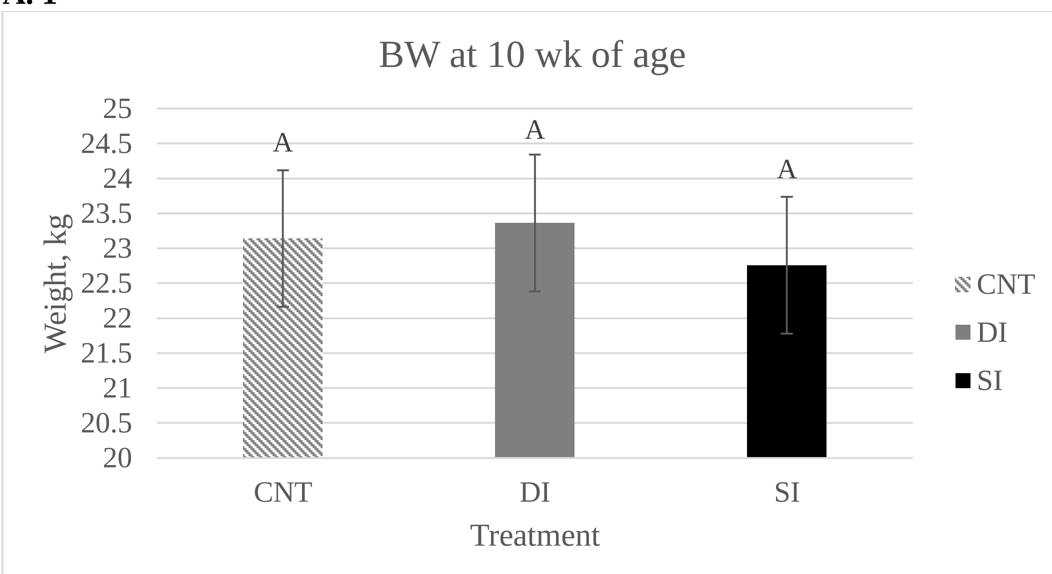
Appendix

A. 1 The effect of single (SI) and double (DI) injections of Improvest (Zoetis Animal Health, Florham Park, NJ) on growth performance.

	Treatment				*P*-values		
Item	CNT[1]	DI	SI	SEM	TRT	Week	TRT*Week
BW, kg							
10	23.141	23.362	22.758	0.979	0.889	-	-
15-25	83.435[y]	87.4714[x]	85.287[x,y]	1.421	0.077	< 0.001	0.522
ADG, kg/d							
1-10	0.3245	0.3294	0.3219	0.013	0.921	-	-
10-25	0.5216[b]	0.5768[a]	0.5574[a,b]	0.051	0.032	< 0.001	0.944

[1]Control
[a-b] Within row, means without a common superscript differ ($P < 0.05$)
[x-z] Within row, means without a common superscript differ ($P < 0.12$)

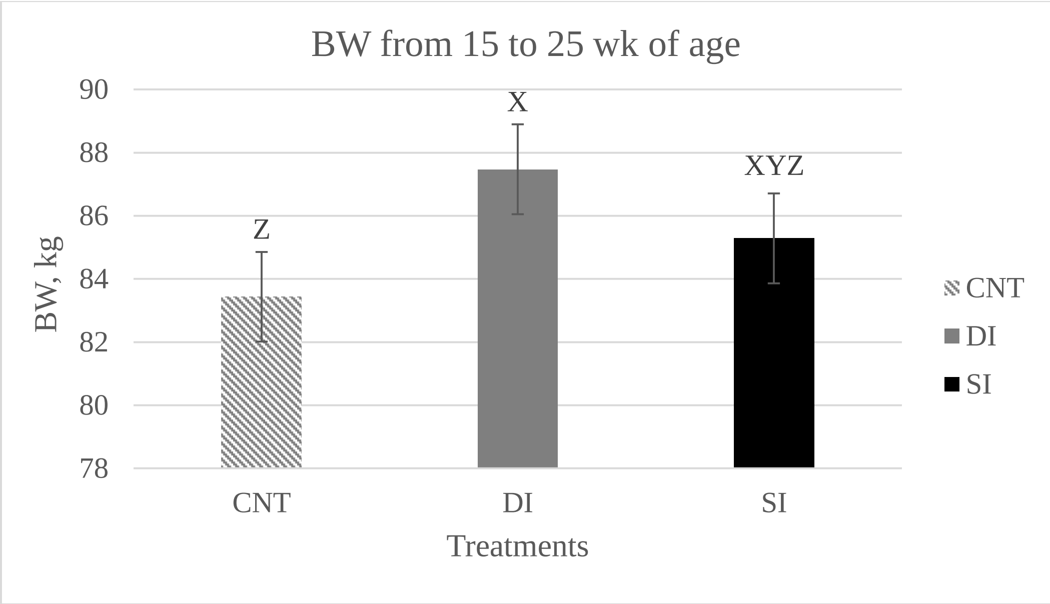
A. 1



A. 1 Body weight at 10 wk of age for each treatment. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. [A-C] Means separated without a common letter differ ($P < 0.05$).

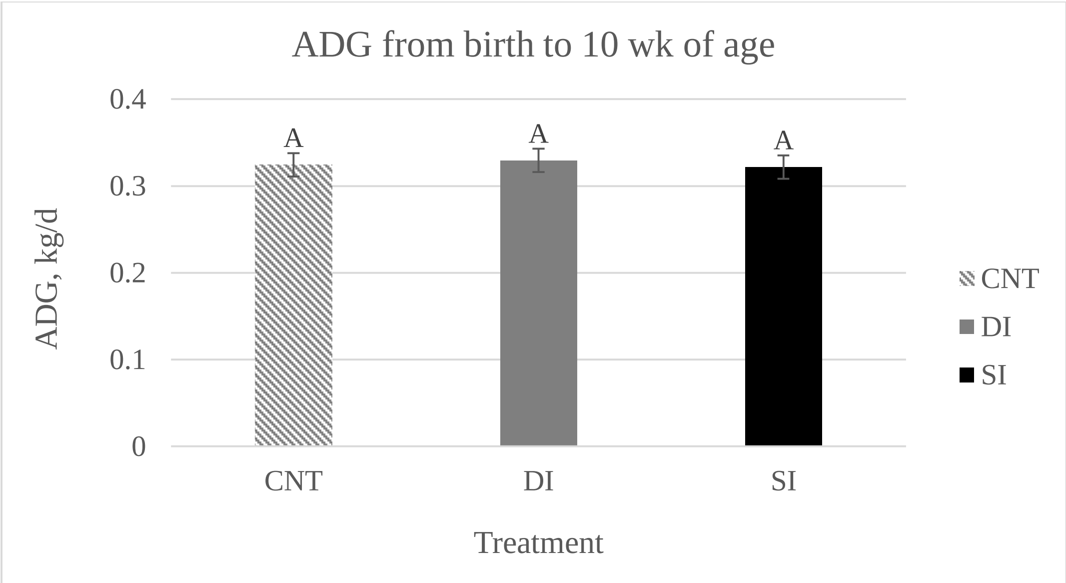
Appendix

A. 2



A. 2 Body weight for each treatment between 15 to 25 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. [X-Z] Means separated without a common letter differ (P < 0.12).

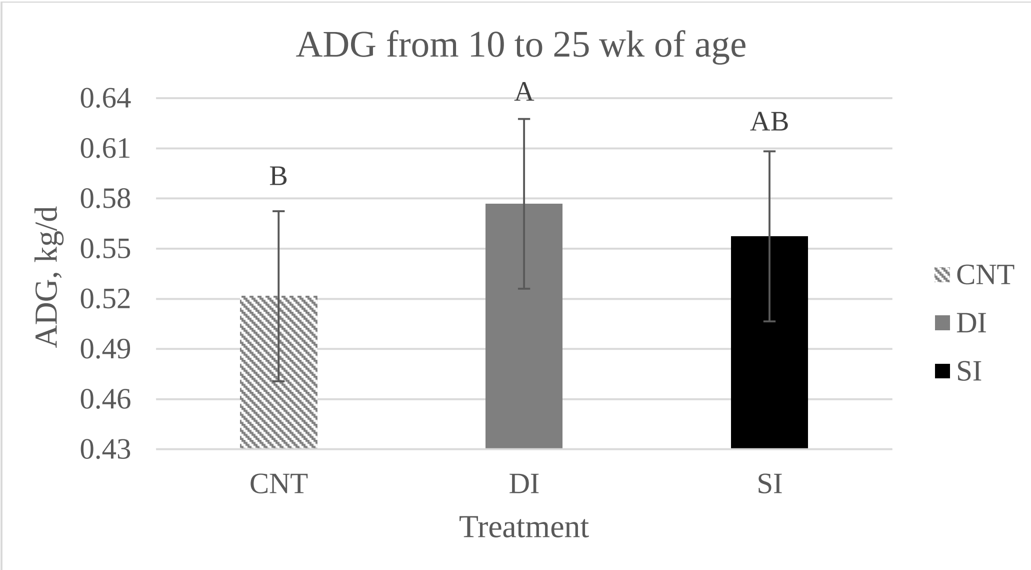
A. 3



A. 3 Average daily gain for each treatment between birth and initiation of the trial period (10 wk of age). Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. [A-C] Means separated without a common letter differ (P < 0.05).

Appendix

A. 4



A. 4 Average daily gain for each treatment between initiation of the trial (10 wk of age) and end of the short-term trial period (25 wk of age). Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. [A-C] Means separated without a common letter differ (*P* < 0.05).

A. 2 The effect of single (SI) and double (DI) injections of Improvest (Zoetis Animal Health, Florham Park, NJ) on estradiol and testosterone concentrations in serum[1].

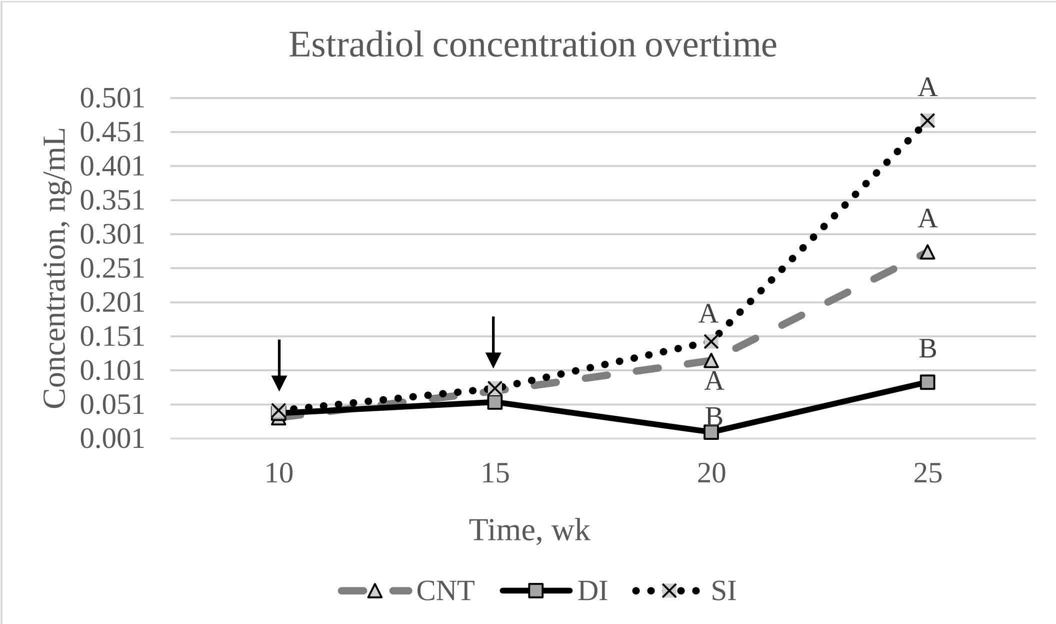
	Treatment				*P* values		
Item	CNT	DI	SI	SEM	TRT	Week	TRT*Week
Estradiol, ng/mL							
10-25 wk	0.1242^{a}	0.0464^{b}	0.1816^{a}	0.024	< 0.001	< 0.001	< 0.001
15 wk	0.0709	0.0545	0.0746	0.015	0.999	-	-
20 wk	0.1157^{a}	0.0106^{b}	0.1435^{a}	0.020	< 0.001	-	-
25 wk	0.2751^{a}	0.0841^{b}	0.4735^{a}	0.083	< 0.001	-	-
Testosterone, ng/mL							
10-25 wk	2.1585^{a}	0.5972^{b}	2.3031^{a}	0.321	< 0.001	< 0.001	< 0.001
15 wk	0.7206	0.8198	0.8773	0.164	0.999	-	-
20 wk	1.3559^{a}	0.0645^{b}	2.1304^{a}	0.387	< 0.001	-	-
25 wk	6.0533^{a}	1.1778^{b}	6.1805^{a}	1.611	< 0.001	-	-
40 wk	1.8830	2.0426	2.7896	0.579	0.473	-	-

[a-c] Within a row, means without common superscript differ (*P* < 0.05)

[1] Log transformation on data was performed for normal distribution, data reported represent the actual mean and standard error.

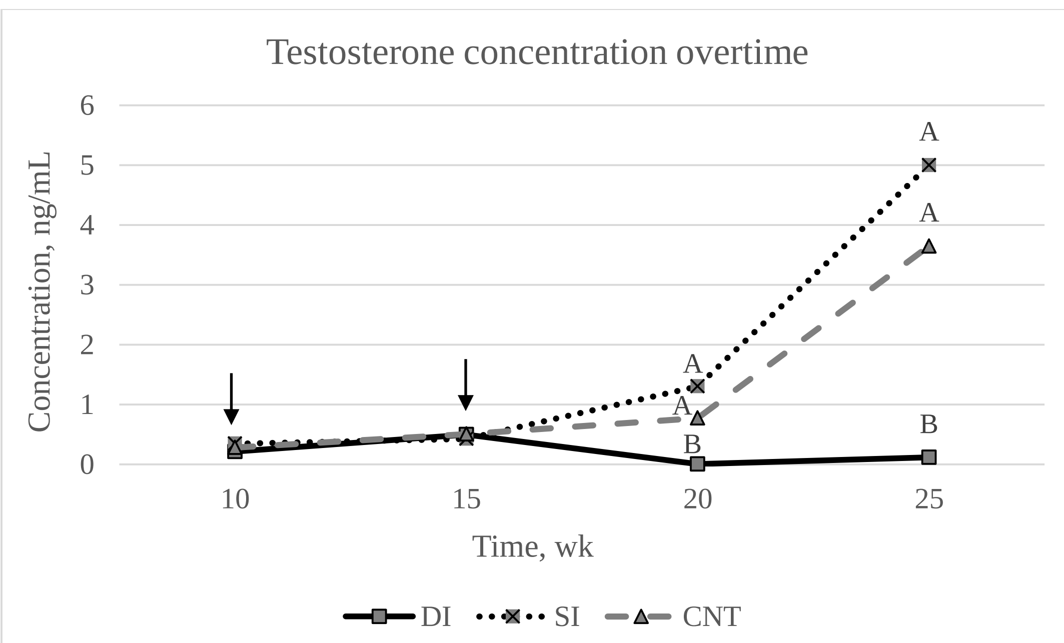
Appendix

A. 5



A. 5 Estradiol concentration in serum for each treatment over time. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Arrows indicate immunization. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means within time without a common letter differ (P < 0.05).

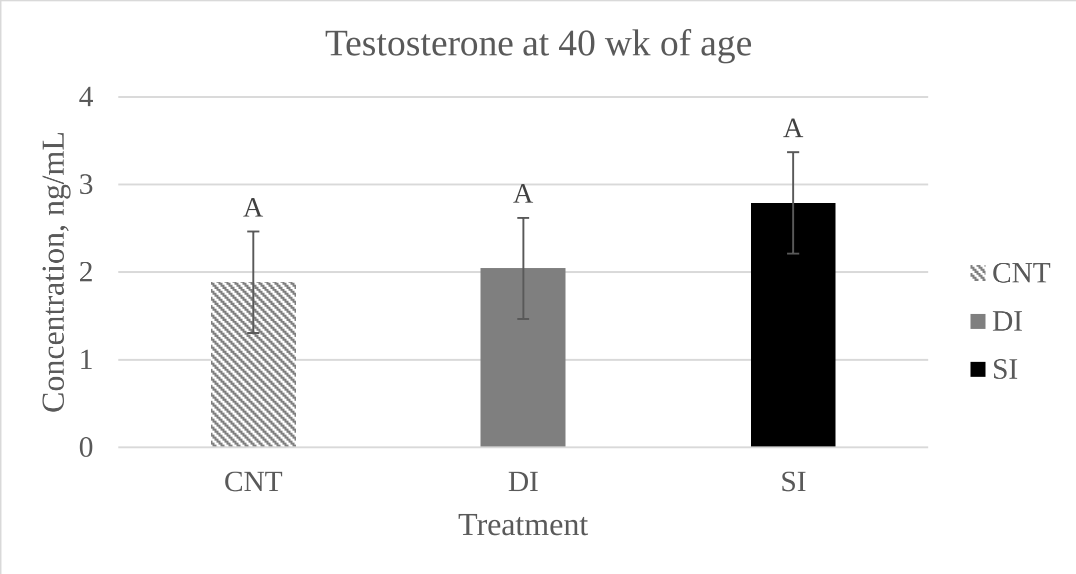
A. 6



A. 6 Testosterone concentration in serum for each treatment over time. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Arrows indicate immunization. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means within time without a common letter differ (P < 0.05).

Appendix

A. 7



A. 7 Testosterone concentration in serum for each treatment at 40 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. [A-C] Means separated without a common letter differ (P < 0.05).

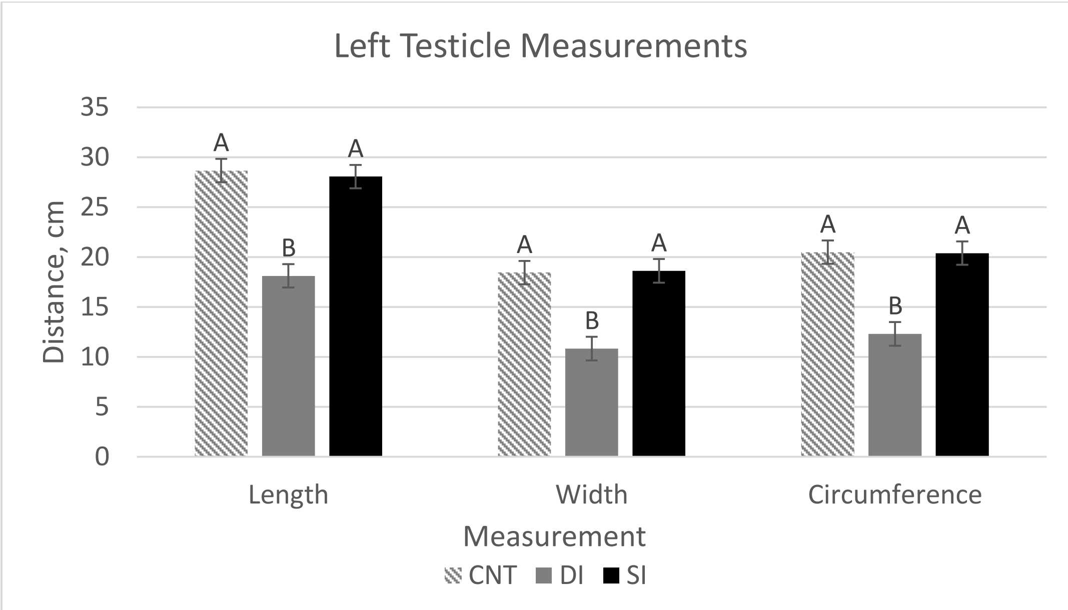
A. 3 The effect of single (SI) and double (DI) injections of Improvest (Zoetis Animal Health, Florham Park, NJ) on testicular measurements.

	Treatment				*P* values
Item	CNT	DI	SI	SEM	TRT
Left Testicle					
Length, cm	28.6597[a]	18.1187[b]	28.0670[a]	1.173	< 0.001
Width, cm	18.4573[a]	10.8373[b]	18.6267[a]	0.660	< 0.001
Circumference, cm	20.5000[a]	12.3167[b]	20.4000[a]	0.657	< 0.001
Weight, g/kg BW	275.6700[a]	68.3610[b]	280.6200[a]	20.268	< 0.001
Right Testicle					
Length, cm	27.9400[a]	17.4413[b]	27.6437[a]	1.112	< 0.001
Width, cm	17.6107[a]	10.7950[b]	17.9917[a]	0.572	< 0.001
Circumference, cm	20.1000[a]	11.8333[b]	20.0500[a]	0.726	< 0.001
Weight, g/kg BW	255.0500[a]	63.1101[b]	254.1600[a]	18.648	< 0.001

[a-c] Within a row, means without common superscript differ (P < 0.05)

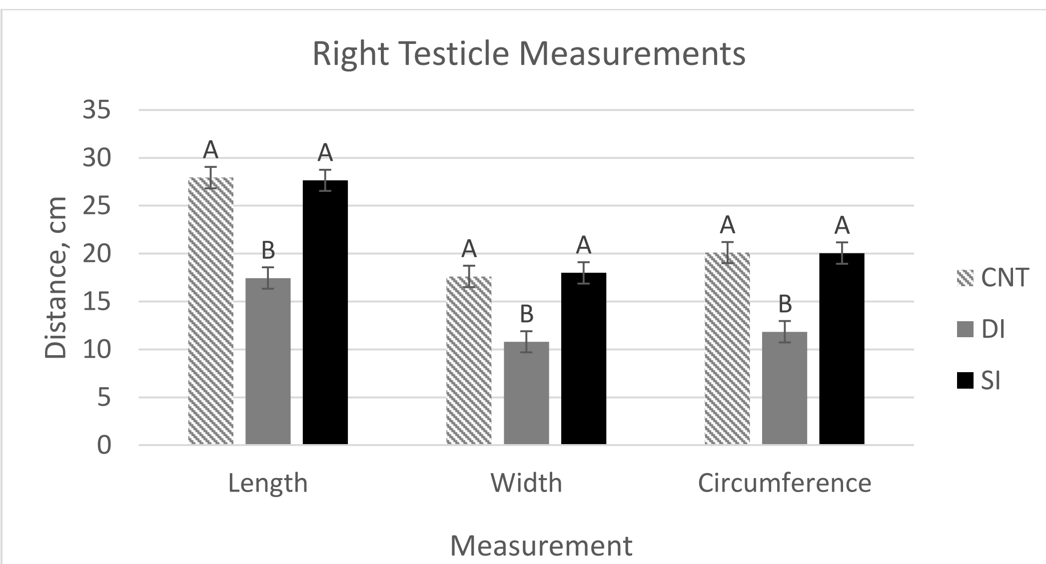
Appendix

A. 8



A. 8 Left testicle measurements for each treatment at 25 wk of age. Measurements included length, width, and circumference were measured on testes without the presence of the epididymides. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. A-C Means within measurement without a common letter differ (P < 0.05).

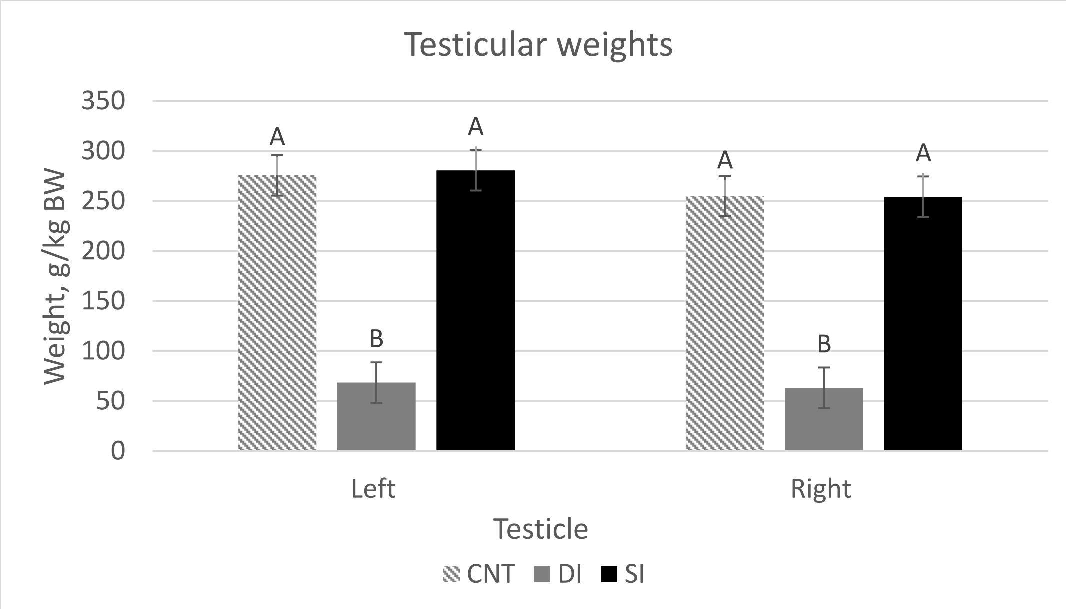
A. 9



A. 9 Right testicle measurements for each treatment at 25 wk of age. Measurements included length, width, and circumference were measured on testes without the presence of the epididymides. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. A-C Means within measurement without a common letter differ (P < 0.05).

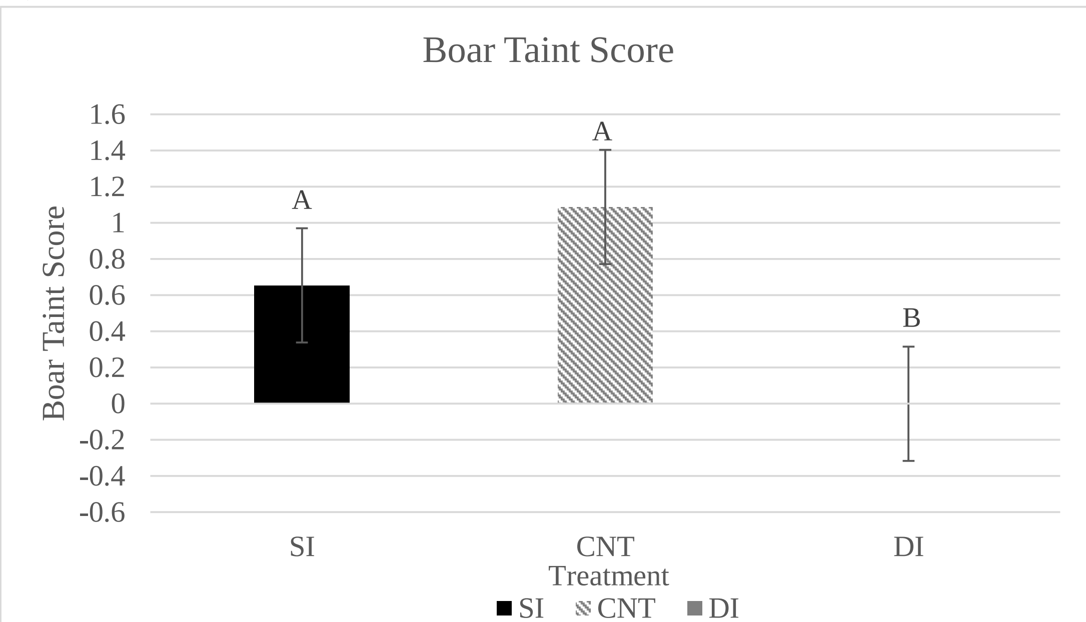
Appendix

A. 10



A. 10 Left and right testicle weights for each treatment at 25 wk of age. Weights were analyzed on a gram per kilogram of BW basis. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means within testicle without a common letter differ ($P < 0.05$).

A. 11



A. 11 Boar taint aroma score for each treatment at 25 wk of age. Boar taint was scored in a boar aroma panel analysis. Any score above zero represents detectable levels of boar taint in heated back fat. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means separated without a common letter differ ($P < 0.05$).

Appendix

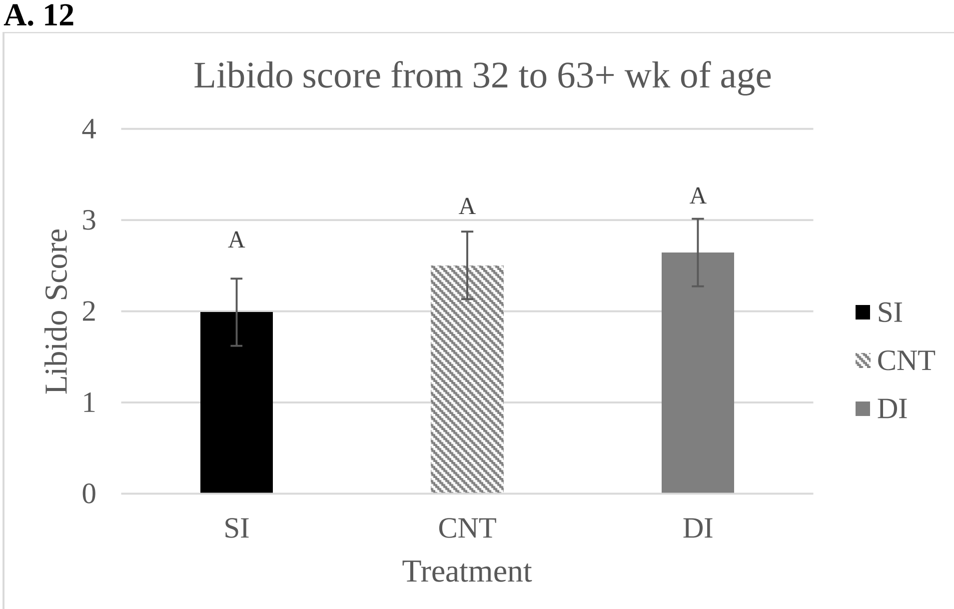
A. 4 The effect of single (SI) and double (DI) injections of Improvest (Zoetis Animal Health, Florham Park, NJ) on libido and semen quality.

	Treatment				*P* values		
Item	CNT	DI	SI	SEM	TRT	Week	TRT*Week
Libido							
32 wk	1.500	1.000	1.500	0.183	0.101	-	-
32-63+ wk	1.990	2.503	2.643	0.370	0.412	0.025	0.713
Semen							
Volume, mL	277.510	316.550	372.690	66.733	0.555	0.284	0.644
Gel weight, g	49.169	60.611	52.002	17.350	0.837	0.299	0.808
Concentration, $x10^6$/mL	272.130^{y}	$344.130^{a, x}$	239.080^{b}	24.873	0.011	0.644	0.644
Total sperm, $x10^9$	75.790	109.300	88.178	20.064	0.409	0.327	0.687
Sperm motility, %	77.500^{y}	86.875^{x}	83.750	2.577	0.066	0.519	0.120

$^{a-c}$ Within a row, means without common superscript differ ($P < 0.05$)

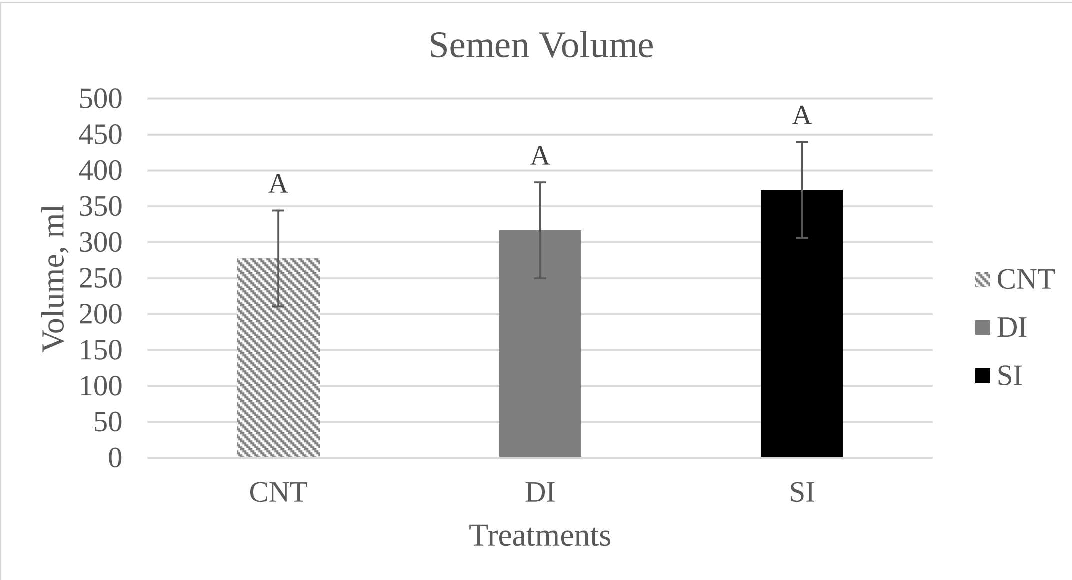
$^{x-z}$ Within a row, means without common superscript differ ($P < 0.12$)

A. 12



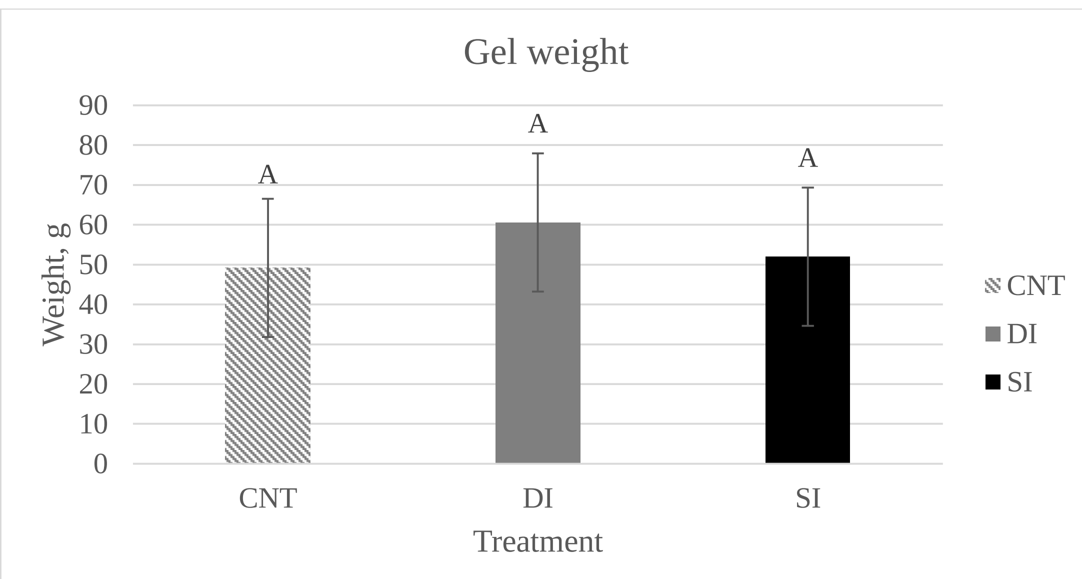
A. 12 Mean libido score for each treatment between 32 and 63+ wk of age. Libido was scored on behavior when the boar was introduced to an artificial sow. Scores ranged from 1 to 5, with 1 representing no libido and 5 representing high libido. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A-C}$ Means separated without a common letter differ ($P < 0.05$).

A. 13



A. 13 Semen volume for each treatment around 60 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means separated without a common letter differ (P < 0.05).

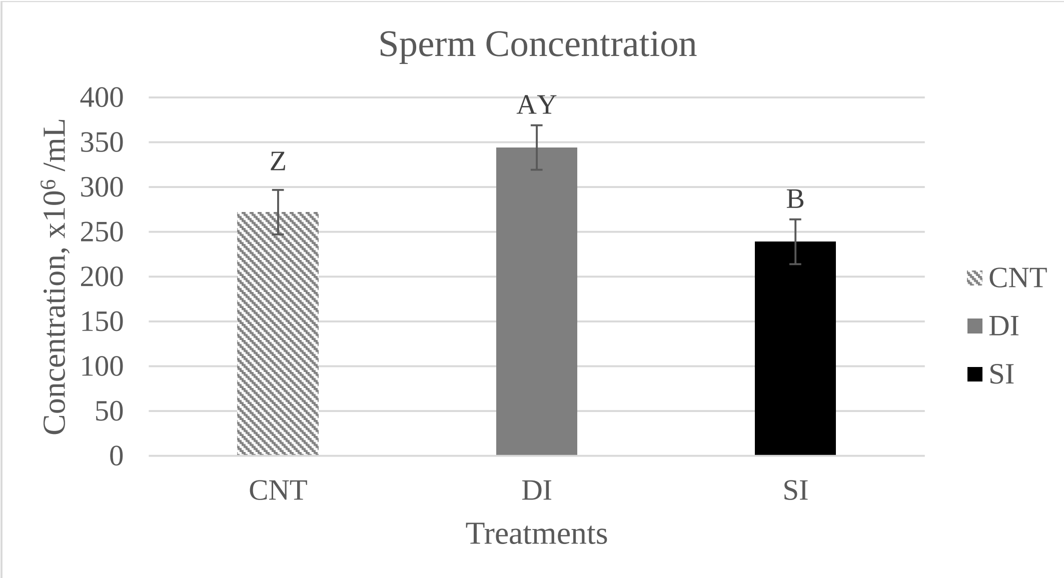
A. 14



A. 14 Gel weight for each treatment around 60 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means separated without a common letter differ (P < 0.05).

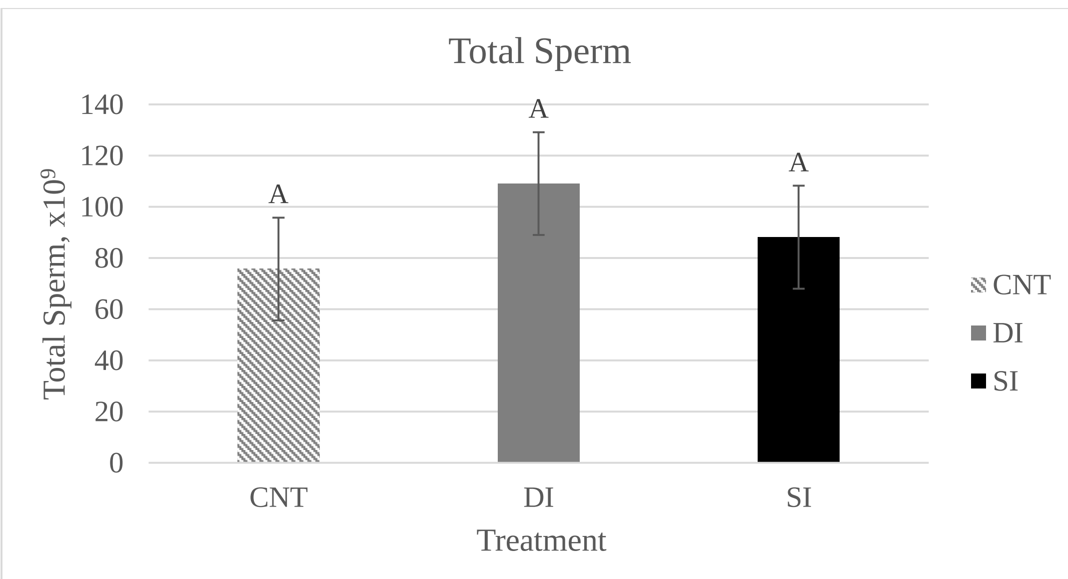
Appendix

A. 15



A. 15 Sperm concentration for each treatment around 60 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means separated without a common letter differ (*P* < 0.05). $^{X\text{-}Z}$ Means separated without a common letter differ (*P* < 0.12).

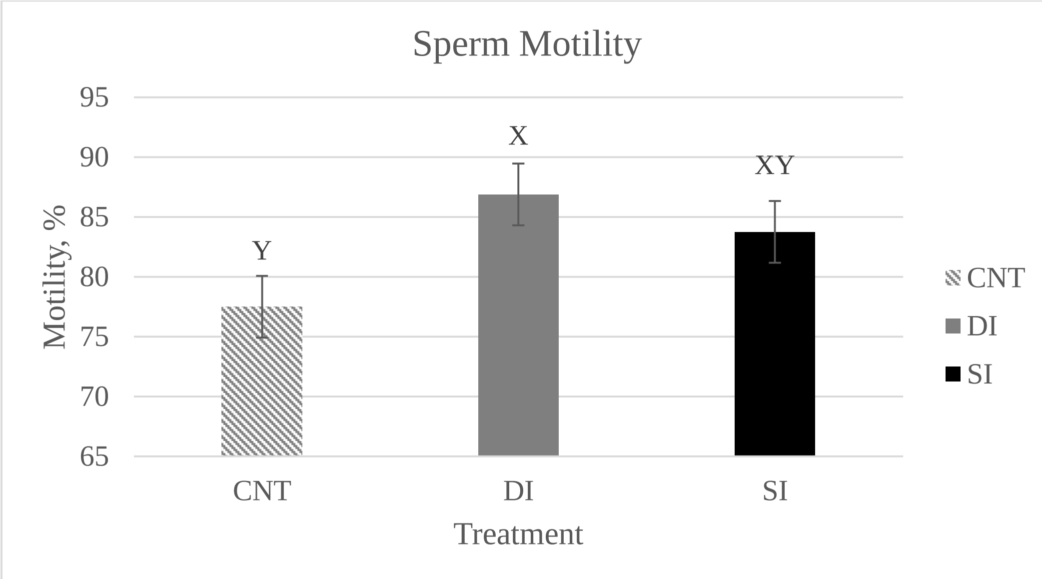
A. 16



A. 16 Total sperm for each treatment around 60 wk of age. Total sperm was calculated by multiplying sperm concentration by semen volume. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{A\text{-}C}$ Means separated without a common letter differ (*P* < 0.05).

<u>Appendix</u>

A. 17



A. 17 Sperm motility for each treatment around 60 wk of age. Pigs that received a single immunization (SI) of Improvest were given the injection at 10 wk of age. Pigs that received a double immunization (DI) of Improvest were given the first injection at 10 wk of age and the second injection at 15 wk of age. Intact control (CNT) did not receive an injection. $^{X\text{-}Z}$ Means separated without a common letter differ (P < 0.12).