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Immunocastration of boars with GnRH-rPE conjugated antigen [1]

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Abstract

Gonadotropin-releasing hormone (GnRH) is a self-antigen and a small molecule that usually could not elicit immune response. To generate an antibody against endogenous GnRH for immunocastration, it is necessary to link GnRH to immuno-carriers for increased immunogenicity. *Pseudomonas aeruginosa* is an opportunistic pathogen often causing infection in domestic animals. The toxicity of *Pseudomonas* is mainly caused by exotoxin A which is an adenosine diphosphate (ADP)-ribosyl transferase. A truncated nontoxic *Pseudomonas aeruginosa* exotoxin A (rPE) modified by gene deletion was conjugated chemically with GnRH, and the GnRH-rPE conjugate was used as an immunogen to immunize boars. The results showed that boars immunized with GnRH-rPE conjugated successfully and induced the generation of anti-GnRH and anti-PE antibodies. The immune response against GnRH resulted in the atrophy of various male reproduction related organs, in conjunction with a significantly decreased serum testosterone concentration to a level similar to castrated boars, which persisted until the end of the study (29 weeks) when boars were ready for release to the market. These results demonstrated that GnRH-rPE conjugates can be used to produce a potential castration vaccine for domestic animals.

Key words: Immunocastration, Gonadotropin-releasing hormone (GnRH), *Pseudomonas aeruginosa* (PE), Boars.

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Introduction

Although non-castrated boars have a better growth efficiency, leaner carcasses than barrows, improved feed conversion and feed efficiency, and thus reduced production costs, the majority of male piglets are still surgically castrated to reduce aggression and sexual behaviors (Rydmher *et al.*, 2010). Besides, the most relevant motivation for castration is the need to reduce the boar taint in the meat of male pigs (Brunius *et al.*, 2011). Testosterone has been suggested to be the cause of boar taint (Bonneau *et al.*, 1992). The surgical castration not only contradicts present attitudes of animal welfare, but also causes infections, stress and growth retardation, reduced carcass quality, and even the loss of animals. To circumvent the disadvantages of surgical castration, the development of a more efficient and suitable method to eliminate boar taint is critical (Brunius *et al.*, 2011).

GnRH, an evolutionarily conserved decapeptide, is produced in the hypothalamus and released to the capillary blood within the median eminence, and eventually binds to the gonadotropes in pituitary. The binding of GnRH to the gonadotropes induces the synthesis and release of LH and FSH from the pituitary (Schally *et al.*, 1973; Braden *et al.*, 1989). In male animals, LH regulates testosterone synthesis and FSH is necessary for the initiation and maintenance of spermatogenesis. Testosterone is needed for spermatogenesis and the development of secondary sexual characteristics including male behaviours such as territorial marking, mounting, and aggressiveness (Mitjana *et al.*, 2020). In female animals, both LH and FSH are required to stimulate the ovarian activities leading to ovulation and successful pregnancy.

Pseudomonas aeruginosa is an opportunistic pathogen, which often causes infection in domestic animals that have their skin barrier breached. The toxicity of *Pseudomonas aeruginosa* is caused primarily by the exotoxin A (PE), an ADP-ribosyl transferase (Hwang *et al.*, 1987). A truncated and modified form of PE has been produced and validated as a more potent immunotoxin (Qaiser *et al.*, 2018). In addition, the *Pseudomonas* exotoxin has been suggested as an excellent biological adjuvant (Fattom *et al.*, 1995); we have also observed that the injection of recombinant detoxicated PE protein (rPE) as a immunostimulatory agent in the vaccine could elicit sufficient immune responses to protect mice from the intoxication by a high dosage of native *Pseudomonas* exotoxin (Shiau *et al.*, 2001).

Since GnRH is a hapten and a self- antigen, GnRH or GnRH analogues must be linked to a suitable carrier molecule in order to obtain immunogenicity for antibody production (Ladd *et al.*, 1988). It has been demonstrated that the active immunization against GnRH could be used as an alternative castration in addition to a surgical method. Immunological suppression of gonadotropin and androgen depletion results in the impairment of reproductive functions in males. The utilization of GnRH as an antigen for immuno-suppression of reproduction has been successfully demonstrated by using analogues conjugating to large molecules such as ovalbumin or tetanus toxin (Talwar and Raghupathy, 1989). The administration of GnRH-carrier induces anti-GnRH antibodies prevents gonadotropin release, and in turn decreases testicular size and inhibits both spermatogenesis and gametogenesis leading to infertility (Mitjana *et al.*, 2020). Anti-GnRH vaccines have been developed for the treatment of hormone-dependent malignancies and non-surgical castration in animals (Adams *et al.*, 1993; Thompson, 2000; Noya *et al.*, 2020). Accordingly, the aim of this study was to immunize the domestic boars with GnRH-rPE conjugate for its immuno-suppression effects on the sex gland repression and potential as a castration vaccine.

Materials and Methods

I. Bacterial strains and plasmid

Escherichia coli strain HB101 was used as the host for the experiments and BL21 (DE3) was used as the host for the expression of recombinant PE. In the pJH4 plasmid construct (Hwang *et al.*, 1987), the structural gene of PE was inserted on a T7 promoter. The PE gene with a deletion in the C-terminal coding for the 533 to 613 amino acid was inserted downstream to a T7 promoter, ribosome binding site and its accompanying ATG initiation codon. Consequently, the production of PE could be induced upon the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG) to drive the expression of T7 RNA polymerase gene that was conferred on the *E. coli* BL 21 (DE3) chromosome under the control of a *lac* promoter.

To produce the recombinant “detoxicated” PE toxin protein, BL21 (DE3) cells carrying the pJH4 plasmid were

grown at 37℃ to an OD_{650} = 0.3. IPTG was added at the final concentration of 1 mM to induce T7 RNA polymerase and the incubation was continued for 2 hrs. Subsequently, the PE gene was turned off by the synthesis of T7 RNA polymerase. The proportion of protein produced by pJH4 induced with IPTG could reach as high as 20% of total *E. coli* protein (Fig. 1). PE protein was then isolated as an inclusion body and solubilized with the refolding kit (Novagen).

II. Conjugation of GnRH to PE

The rPE protein synthesized by *E. coli* was used as a carrier protein for chemical conjugation. A modified GnRH decapeptide with lysine substitutions on amino acid position six (D-Lys^6-LH-RH) (Sigma) was conjugated to purified rPE at 1:5 (W/W) by chemical method using the amine-carboxylic conjugation kit (Pierce). The success of conjugation was determined by the molecular weight shift and by the Western blot using anti-GnRH and anti-PE antibody on 13% SDS-PAGE gel, respectively (Fig. 2). The GnRH-rPE conjugate was then used as an immunogen emulsified in the Complete Freund Adjuvant (CFA, Gibco) and Incomplete Freund Adjuvant (IFA, Gibco).

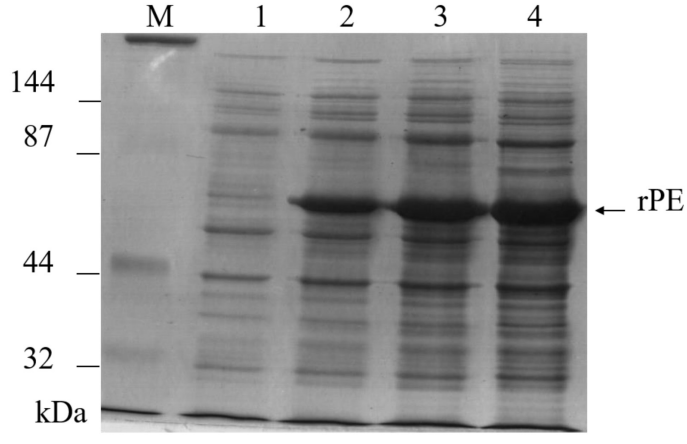

Fig. 1. The expression of rPE protein in *Escherichia coli* strain BL21 (DE3) after IPTG induction at 1 to 4 hrs. (lane 1-4). Lane M indicates the molecular weight marker.

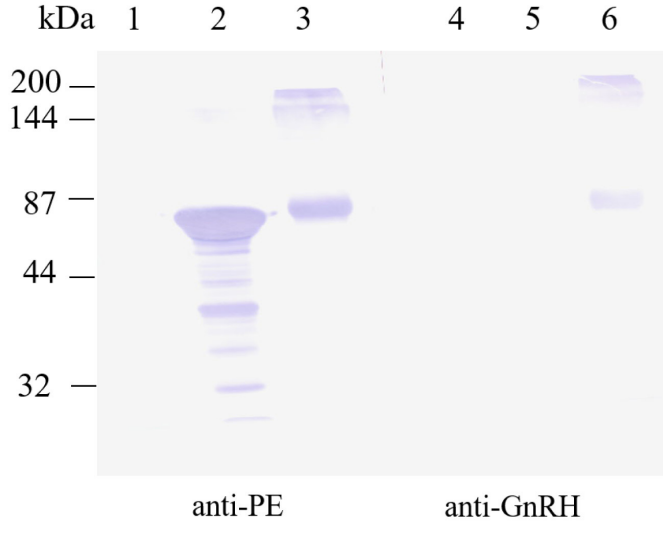

Fig. 2. Identification of the rPE and GnRH-rPE conjugate by Western blotting with antibodies to PE (lanes 1 - 3) and GnRH (lanes 4 - 6), respectively. Lanes 1 and 4 were negative controls. Lanes 2 and 5 were rPE proteins. Lanes 3 and 6 were GnRH-rPE conjugates.

III. Vaccination and serum sample collection

Sixteen crossbred (Landrace × Yorkshire × Duroc) boars were randomly divided into two groups. Boars in the control group were mock vaccinated with 1 mL PBS in CFA at 9 weeks old, and were boosted with 1 mL PBS in IFA at 17 weeks old. Boars of the vaccinated group were injected subcutaneously with 1 mg of GnRH-rPE conjugate dissolved in 1 mL of PBS and emulsified with equal volume of CFA. Booster dose in IFA with the same vaccination schedule was

performed as those of the control group.

Blood samples were collected every week from the jugular vein for determination of serum testosterone concentrations and the titer of antibodies against GnRH and PE until the end of 29 weeks of age. Sera were obtained by centrifugation separation (1,300 × g for 10 min) and stored at -20℃ before assay.

IV. Serum testosterone assays

The concentrations of serum testosterone were measured by radioimmunoassays (RIAs) according to the method described by Li *et al.* (1987).

V. Detection of Antibody to GnRH and PE

Antibody titers to GnRH and PE in boars immunized against GnRH-rPE were evaluated by ELISA. Each well of the microtiter plates (Nunc 467466) was coated with 100 μL rPE protein conjugated with keyhole limpet hemocyanin (KLH) at a concentration of 5 μg/mL in 0.1 mL of 0.05 M carbonate bicarbonate coating buffer (pH 9.6) for overnight at 4℃. Thereafter, the plates were washed with PBS/T (0.5% Tween-20 in PBS, v/v), and blocked with a 0.5% (w/v) bovine serum albumin (BSA) solution in PBS overnight and then stored at 4℃.

The plates were washed twice with PBS/T before use. Each serum sample was diluted 1,000 and 4,000 folds, then added to a GnRH-KLH and a rPE-KLH coated well, and incubated at room temperature for two hours. The plates were washed four times with PBS/T to remove the serum sample and incubated with the goat-anti-mouse IgG (Sigma, diluted 1:10,000 in PBS) for two hrs at room temperature. Plates were washed four times with PBS/T before adding the alkaline phosphatase. Reaction was performed by incubating at 37℃ for 30 min. After the addition of chromogens, the reaction was terminated by the addition of 3 N NaOH and was assayed in a microtiter plate reader (Molecular Device) to measure the absorption at 405 nm.

VI. Sex gland weights and preparations

All boars were slaughtered at the end of 29 week of age. Testes and accessory reproductive organs (epididymis, prostate seminal vesicles, and bulbo-urethral glands) were dissected, weighted, and immediately fixed in Bouin's solution (HT10132, Sigma -Aldrich) for the subsequent histological examination. Fixed tissues were embedded in the paraplast, sectioned into 5 μm slices, mounted individually on microscope slides, and stained with H & E for the histological evaluation under a light microscopy.

VII. Statistical analysis

Differences between groups were tested by one-way analysis of variance, and pairwise multiple comparisons were tested with the Chi-square method. The level of significance was $P < 0.05$.

Results

I. Antibody titer and testosterone concentration

Boars were immunized against GnRH by injections at weeks 9 and 17. All the GnRH-rPE immunized boars produced antibodies against GnRH (A) and PE (B) as evidenced by the serum antibody titers after two vaccinations (Fig. 3). The serum antibody titers of the GnRH-rPE immunized boars dropped slowly after booster immunization. No antibody titer against GnRH and PE was detected in the serum of control boars that were mock immuned injected with PBS/CFA (Fig. 3). This high antibody titer also coincided with the decrease of serum testosterone concentrations to a non-detectable level (Fig. 4), indicating a success of neutralization of endogenous GnRH. The mean of serum testosterone concentration decreased to < 0.1 ng/mL in GnRH-rPE immunized boars by week 17 and consistently remained at lower levels than that of the boars in the control group until the end of trial (Fig. 4).

II. The development of reproductive organs after immunization

GnRH-rPE immunized boars exhibited smaller sex glands as shown in Table 1 and in Fig. 5. The weight of testes, epididymis, seminal vesicles, prostate glands, and bulbo-urethral glands were significantly decreased, with a range from 90.8% for bulbo-urethral glands to that of 71.4% for seminal vesicles (Table 1). The size and weight of testis of immunized animals were significantly less than those of the control bars. Thus, the normal development of the

reproductive system in GnRH-rPE treated boars was blocked by active immunization against GnRH.

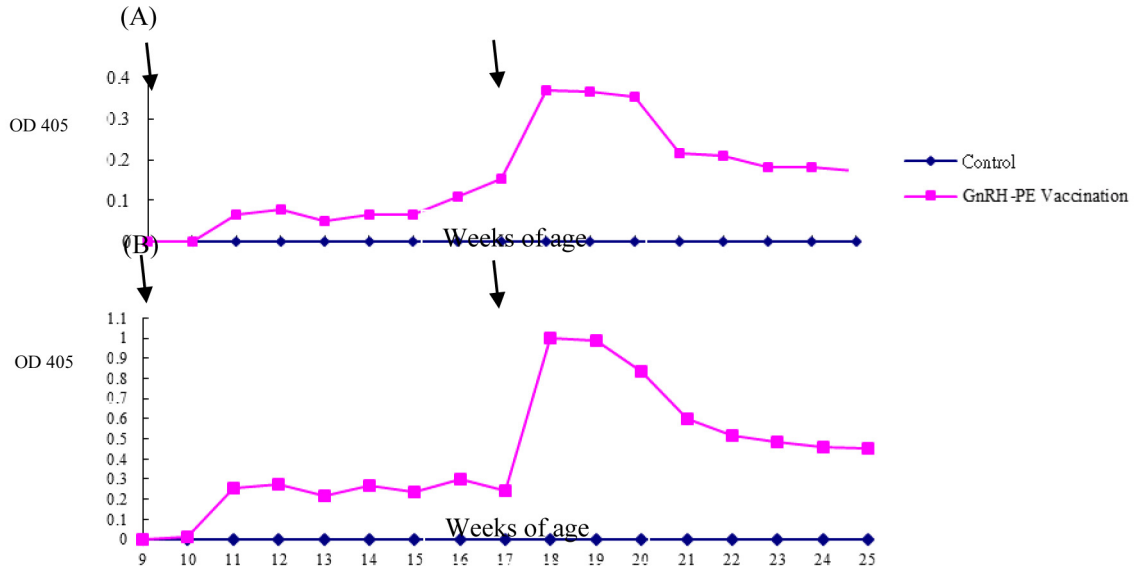

Fig. 3. Detection of GnRH and PE-reactive antibodies in the GnRH-rPE vaccinated and control boars by ELISA. Changes of anti-GnRH (A) and anti-PE (B) antibody titers at 1: 1,000 (A) and 1: 4,000 (B), respectively. Arrows indicate the time of primary and booster immunization.

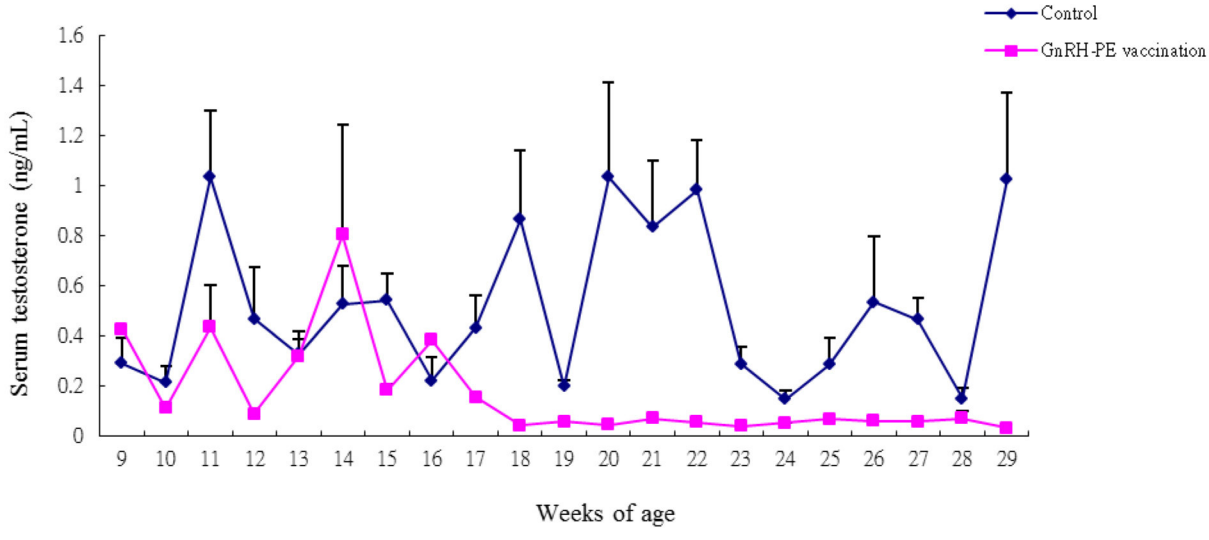

Fig. 4. Changes of serum testosterone concentration (ng/mL) in GnRH-rPE immunized and control boars. All animals were vaccinated at the age of 9 and 17 weeks .Values represent means ± S.E of animals in the treatment group and in the control group. The intra- and inter-assay coefficients of variation (CV) of testosterone was 4.0% and 4.7%, respectively.

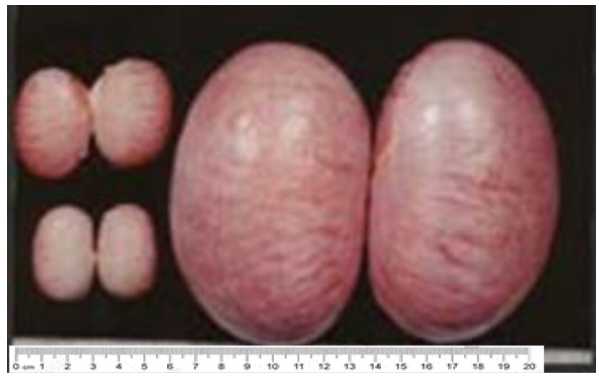

Fig. 5. Testes of the control (right) and GnRH-rPE immunized boars (left) at 29 week of age.

Table 1. The comparison of weight of testes, epididymis, seminal vesicles, prostate glands, and bulbo-urethral glands between the GnRH-rPE vaccinated and the control LYD boars at 29 week of age

	Mean ± SE Weight (g) of reproductive organs		
	Vaccinated	Control	% of suppression
Number of pigs	8	8	
Testes	53.3 ± 27.5[a]	345.3 ± 90.0[b]	84.6
Epididymis	7.2 ± 8.7[a]	145.0 ± 50.3[b]	81.2
Seminal vesicles	3.2 ± 1.5[a]	75.8 ± 27.1[b]	71.4
Prostate glands	1.9 ± 1.1	11.2 ± 9.5	83.1
Bulbo-urethral glands	10.6 ± 5.8[a]	115.2 ± 34.4[b]	90.8

[a, b] Means within the same row with different superscripts differ significantly ($P < 0.05$).

III. Histologic analysis

Histologic evaluations with the cross-sections of testis showed remarkable differences between the control and GnRH-rPE immunized boars (Fig. 6). In the control boars, the interstitial tissue was completely filled with distinct Leydig cells, and seminiferous tubules showed various stages of cycle of the seminiferous epithelium. The completion of spermatogenesis was evidenced by the presence of elongated spermatids in the lumen.

In contrast, as indicated by histologic observations, GnRH-rPE immunized boars showed a thickened basement membrane and reduced diameter in the seminiferous tubules. Although some pachytene spermatocytes were present in the seminiferous tubules of GnRH-rPE immunized boars, the spermatocytes appeared to undergo degenerating. Besides, motile semen samples could not be obtained from any of the GnRH-rPE immunized boars. There were no elongated spermatids present in the lumen. The nuclei of the Sertoli cells had a shrunk morphology and their cytoplasms were vacuolated while the Leydig cells had an atrophic morphology. Accordingly, the spermatogenesis was impaired in the testis of GnRH-rPE immunized boars. These findings suggest the potential of GnRH-rPE immunization as an alternative method to castration.

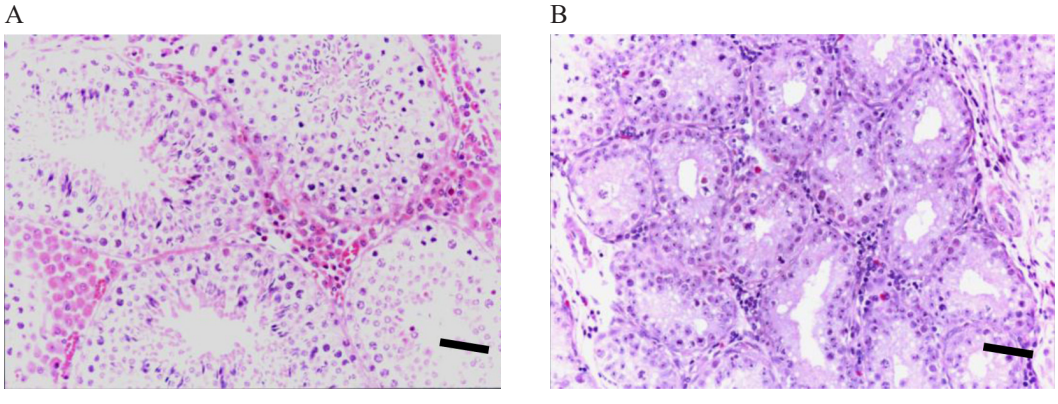

Fig. 6. Histological analysis (cross section) of testis from the control boars and the GnRH-rPE immunized LYD boars at 29 weeks of age. (A) Control boars, the completion of spermatogenesis was observed by the presence of elongated spermatids in the lumen. (B) GnRH-rPE immunized boars are absent with elongated spermatids in all tubules. The diameter of the seminiferous tubules was remarkably different between the control and immunized boars. Magnifications × 200. Scale bars = 100 μm.

Discussion

Active and passive immunization against the hypothalamic decapeptide GnRH have been proven as a potential tool for practical, on-farm applications in reproductive performance control as well as a very powerful tool for studying basic aspects of the hypothalamic-pituitary-gonadal axis (Thompson, 2000; Squires *et al.*, 2020). Reasons for such immunocastration include improvement of meat and carcass characteristics for cattle, sheep, goats, and swine; improvement on feed efficiency

relative to castrates in those species; reduction in male aggressive behaviors; reduction in male-associated odors in goats and swine; and fertility neutralization in pet species. Although application as a fertility control agent in men is unlikely, there were renewed interests in active immunization against GnRH for treating prostate cancers and related sex hormone-dependent disorders (Thompson, 2000)

GnRH is of particular importance, if completely neutralized, the fertility axis can be rendered totally ineffective (Goericke-Pesch, 2017). Since GnRH itself is too small for sufficient immunogenicity to induce antibodies against itself, a unique approach to fool the animal's immune system to recognize endogenous GnRH as foreign molecule can be achieved. GnRH has to be conjugated to large, non-self carrier molecules to become immunogenic. Commonly used carrier proteins include KLH (Goubau *et al.*, 1989), bovine serum albumin (BSA) (Fraser and Gunn, 1973), ovalbumin (Hoskinson *et al.*, 1990), glutaraldehyde (Hotzel *et al.*, 1997), toxoids (diphtheria, tetanus, or cholera) (Ladd *et al.*, 1990; Stevens, 1993), which in conjunction with an appropriate adjuvant can produce an effective immune response (Stevens, 1993). A commercial GnRH vaccine based on the GnRH-ovalbumin was available for ruminants (Adams *et al.*, 1993; Thompson, 2000; Karakuş *et al.*, 2013). In this study we used PE as the carrier protein to investigate the effect of GnRH-rPE on antibody production and testosterone levels, together with the efficacy of the conjugate in gonadal atrophy and fertility impairment. The results showed that the GnRH-rPE conjugate did induce specific antibodies against GnRH by vaccination. We also found that vaccination at 9 week of age, which is the early stage of the porcine lifespan, significantly inhibited the development of reproductive organs such as testis, epididymis, seminal vesicles, prostate glands and bulbo-urethral glands. Most important, the function of spermatogenesis was completely eliminated, and the synthesis of testosterone was also dramatically prevented. These effects indicated the immunoneutralization of GnRH by the induction of sufficient amounts of anti-GnRH antibody that was able to block GnRH in the transmitting path between the hypothalamus and pituitary.

In fact, novel uses of bacterial toxins as immune carrier, such as tetanus (Davis *et al.*, 1987), diphtheria (Allison and Gregoriardis, 1974), cholera (Peirre *et al.*, 1992) have been demonstrated that conjugation of a weak immunogen such as GnRH to bacterial toxins was a very promising approach. PE toxin is a single chain toxin with three major structural domains that inhibit protein synthesis in eukaryotic cells by catalysing ADP ribosylation of elongation factor 2. In addition, the induction of anti-PE antibody had a protective effect on mice against intoxication of the native *Pseudomonas* exotoxin A. Although we did not perform the exotoxin A intoxication experiment in boars, the vaccination of GnRH-rPE conjugate is very likely might provide the similar additional advantage to protect farm animals against *Pseudomonas* infection.

Adjuvants are commonly used in vaccine preparations to increase the immunogenicity of antigens (Charerntantanakul, 2020). The classic choice of adjuvant is FCA, an oil emulsion containing killed Mycobacterium components. The most successful GnRH immunization protocols have included the use of FCA. In contrast to FCA, boars immunized with alumina gel did not produce significant antibody titers (Donald and Thompson, 2000). However, FCA is not useful from a commercial standpoint due to its interference in tuberculin testing and the formation of undesirable lesions at the sites of injection in many species (Ferro and Stimson, 1998). Meloen *et al.* (1994) reported that the use of CFA/IFA in the castration vaccine formula had no systemic or adverse effects at the injection site in any of the treated animals. However, the animals treated with Freund's adjuvant in this study developed ulceration and abscesses at the site of injection.

The question remains of how GnRH is neutralized. Theoretically, free peptides could slowly diffuse out of the adjuvant and act as an agonist for GnRH, thus down-regulating its receptors on the pituitary and switching off reproductive functions. This mechanism would lead to an early disappearance of testosterone in serum. A second mechanism could be the induction of immune responses to GnRH-producing cells in the hypothalamic area and causing damage to GnRH-producing neurons. An additional mechanism could be the antibody directly reacted with GnRH when the GnRH molecules passed through the blood from the hypothalamic GnRH-producing cells to interact with the receptors on the pituitary (Donald and Thompson, 2000; Xu *et al.*, 2018).

Although the precise mechanism is unknown, our results showed the prospects for the development of a new generation, reliable immunomodulation vaccine that can be applied to endocrine and immune regulatory processes. This will be of importance to modulate the dysfunction in endocrine or immune regulation. The advantages of the anti-fertility vaccine against GnRH are the absence of need for the surgical removal of the testes and cost-effectiveness. The other aspect is to prevent prostatic and testicular carcinomas that frequently occur in aged male dogs and cats.

In most studies done with active production of antibodies against GnRH, endocrine changes as well as morphological

changes of gonads have been reported (Sad *et al.*, 1993). Active immunization against GnRH led to the suppression of testicular functions including steroidogenesis and spermatogenesis in boars. Serum testosterone levels closely followed the testicle size. The testicle size is directly proportional to its function. The role of testosterone in the qualitative and quantitative maintenance of both spermatogenesis and testicular morphologic integrity has been established.

Active immunization against GnRH is potentially useful as a nonsurgical method for the fertility regulation of boars. These results and several earlier reports of studies in laboratory animals indicated that this immunological birth control strategy is safe and the induced infertility is reversible (Donald and Thompson, 2000).

In conclusion, the results suggested that immunocastration is a potential tool for management in the pig industry. The results suggest a potential application using immunocastration as a substitute for surgical castration in the pig industry.

References

Adams, T. E., C. A. Daley, B. M. Adams and H. Sakurai. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin - releasing hormone effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. 71: 811-817.

Allison, A. C. and G. Gregoriardis. 1974. Liposomes adjuvant. Nature 252: 252-256.

Bonneau, M., M. Denmat, J. C. Vaudelet, J. R. Veloso Nunesa, A. B. Mortensenc and H. P. Mortensenc. 1992. Contribution of fat androstenone and skatole levels to boar taint: II. Eating quality of cooked hams. Livestock Prod. Sci. 32: 81-88.

Braden, T. D., B. E. Hawes and P. M. Conn. 1989. Synthesis of GnRH receptors by gonadotrope cell cultures. Endocrinology 125: 1623-1629.

Brunius, C., G. Zamaratskaia, K. Andersson, G. Chen, M. Norrby, A. Madej and K. Lundstrom. 2011. Early immunocastration of male pigs with Improvac®: effect on boar taint, hormones and reproductive organs. Vaccine 29: 9514-9520.

Charerntantanakul, W. 2020. Adjuvants for swine vaccines: Mechanisms of actions and adjuvant effects. Vaccine 38: 6659-6681.

Davis, D., A. Davies and G. Gregoriadis. 1987. Liposomes as adjuvants with immunoporified tetanus toxoid: the immune response. Immunol. Letters 14: 341-348.

Fattom, A., X. Li, Y. H. Cho, A. Burns, A. Hawwari, S. E. Shepherd, R. Coughlin, Scott. Winston and R. Naso. 1995. Effect of conjugation methodology, carrier protein, and adjuvants on the immune response to *Staphylococcus aureus* capsular polysaccharides. Vaccine 3: 1288-1293.

Ferro, V. A. and W. H. Stimson. 1998. Investigation into suitable carrier moleculars for use in an anti-gonadotrophin releasing hormone vaccine. Vaccine 16: 1095-1102.

Fraser, H. M., and A. Gunn. 1973. Effects of antibodies to luteinizing hormone-releasing hormone in the male rabbit and on the rat oestrous cycle. Nature 244: 160-161.

Goericke-Pesch, S. 2017. Long-term effects of GnRH agonists on fertility and behaviour. Reprod. Domest. Anim. 52 (Suppl.) 2: 336-347.

Goubau, S., D. W. Silversides, A. Gonzalez, B. Laarvelda, R. J. Mapletoft and B. D. Murphy. 1989. Immunisation of sheep against modified peptides of gonadotropin releasing hormone conjugated to carriers. Domest. Anim. Endocrinol. 6: 339-347.

Hoskinson, R. M., R. D. Rigby, P. E. Mattner, V. L. Huynh, M. D'Occhio, A. Neish, T. E. Trigg, B. A. Moss, M. J. Lindsey and G. D. Coleman. 1990. Vaxstrate: an anti-reproductive vaccine for cattle. Aust. J. Biotechnol. 4: 166-170.

Hotzel, M. J., A. Caraty and G. B. Martin. 1997. Effects of nutrition on testicular growth in mature Merino rams actively immunized against GnRH. J. Reprod. Fertil. 110: 307-313.

Hwang, J., D. J. Fitzgerald, S. Adhya and I. Pastanet. 1987. Functional domains of *Pseudomonas* exotoxin identified by deletion analysis of the gene expressed in *E. coli*. Cell 48: 129-136.

Karakuş, F., A. Yılmaz, B. Hakan, K. Stormo and H. Ulker. 2013. The effectiveness of recombinant OL fusion protein (ovalbumin-LHRH-7) in suppressing reproductive functions when injected in single-dose vaccination protocols with different adjuvants. Anim. Reprod. Sci. 138: 228-232.

Ladd, A., G. Prabhu, Y. Y. Tsong, T. Probst, W. Chung and R. B. Thau. 1988. Active immunonization against GnRH combined with androgen supplementation is a promising antifertility vaccine for males. Am. J. Reprod. Immunol. Microbiol. 17: 12112-12117.

Ladd, A., Y. Y. Tsong, J. Lok and R. B. Thau. 1990. Active immunization against LHRH: I. Effects of conjugation site and dose. Am. J. Reprod. Immunol. 22: 56-63.

Li, P. H., H. M. Chian and T. T. Hsu. 1987. Relationships between serum testosterone concentrations and semen quality in Landrace and Duroc boars housed in a tropical area. J. Chinese Soc. Anim. Sci. 16: 31-39.

Meloen, R. H., J. A. Turkstra, H. Lankhof, W. C. Puijk, W. M. Schaaper, G. Dijkstra, C. J. Wensing and R. B. Oonk. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine 12: 741-746.

Mitjana, O., C. Bonastre, T. Tejedor, L. Garza, Á. Latorre, B. Moreno and V. Falceto. 2020. Immuno-castration of female and male pigs with anti-gonadotrophin releasing hormone vaccine: Morphometric, histopathological and functional studies of the reproductive system. Anim. Reprod. Sci. 221: 106599. doi:10.1016/j.anireprosci.2020.106599.

Noya, A., G. Ripoll, I. Casasús and A. Sanz. 2020. Effects of immunocastration performed at two live weights on the growth physiology, temperament and testicular development of feral beef bulls. Anim. Sci. J. 91: e13307. doi: 10.1111/asj.13307.

Peirre, P., O. Denis, H. Bazin, E. M. Mbella and J. P. Vaerman. 1992. Modulation of oral tolerance to ovalubin by cholera toxin ant its ß- subunit. Eur. J. Immunol. 22: 3179-3182.

Qaiser, H., F. Aslam, S. Iftikhar and A. Farooq. 2018. Construction and recombinant expression of *Pseudomonas aeruginosa* truncated exotoxin A in *Escherichia coli*. Cell Mol. Biol. 641: 64-69.

Rydmher, L., K. Lundstrom and K. Andersson. 2010. Immunocastration reduces aggressive and sexual behavior in male pigs. Animal 4: 965-972.

Sad, S., V. S. Chauhan, K. Arunan and R. Raghupathy. 1993. Synthetic gonadotrophin-releasing hormone (GnRH) vaccines incorporating GnRH and synthetic T-helper epitopes. Vaccine 11: 1145-1156.

Schally, A. V., A. Arimura and A. J. Kastin. 1973. Hypothalamic releasing hormones. Science 179: 341-350.

Shiau, J. W., T. K. Tang, Y. L. Shih, C. Tai. Y. Y. Sung, J. L. Huang, and H. L. Yang. 2001. Mice immunized with DNA encoding a modified *Pseudomonas aeruginosa* exotoxin A develop protective immunity against exotoxin intoxication. Vaccine 19: 1106-1112.

Squires, E. J., C. Bone and J. Cameron. 2020. Pork production with entire males: directions for control of boar taint (review). Animals 10: 1665; doi:10.3390/ani10091665.

Stevens, V. C. 1993. Vaccine delivery systems: potential methods for use in antifertility vaccines. Am. J. Reprod. Immunol. 29: 176-188.

Talwar, G. P. and R. Raghupathy. 1989. Anti-fertility vaccines. Vaccine 7: 97-101.

Thompson, D. L. 2000. Immunization against GnRH in male species (comparative aspects). Anim. Reprod. Sci. 60-61: 459-469.

Xu, M., C. Xu, F. Liu, X. Shen, J. Meng, H. Chen, J. Yang, P. Zhou, R. Gao and S. Gan. 2018. Effects of active immunization with newly modified GnRH peptides on spermatogenesis and production performance of Holstein bulls. Biol. Reprod. 99: 461-472.

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應用激性腺素釋放素－重組綠膿桿外毒素接合抗原進行 LYD 公豬之免疫去勢 (1)

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摘　要

激性腺素釋放素 (Gonadotropin-releasing hormone, GnRH) 是一種小分子自體抗原，通常不會誘發免疫反應。本研究將 GnRH 聯結免疫携帶蛋白以產生對抗 GnRH 之抗體。綠膿桿菌是一種機會型病原菌，其毒性主要源自外毒素 A，以基因剔除技術去除毒性之綠膿桿菌外毒素 (*Pseudomonas* exotoxin, PE) 可作為攜帶蛋白質，應用化學法接合 GnRH 成為重組型 GnRH-rPE，可供為疫苗進行公豬之免疫去勢。本研究於 LYD 公豬第 9 與 17 週齡時注射免疫去勢疫苗，並於第 29 週齡試驗結束時犧牲公豬，進行組織學與血清學等檢測。研究結果顯示，接種重組型 GnRH-rPE 去勢疫苗的公豬成功誘發分別對抗 GnRH 及 PE 的抗體，並導致公豬之相關生殖器官萎縮，血清中睪固酮含量亦降低至如同外科法去勢公豬的水準，並持續至 29 週齡試驗結束準備上市階段。本研究結果證明，重組型 GnRH-rPE 具有潛力開發成為家畜用之免疫去勢疫苗。。

關鍵詞：免疫去勢、激性腺素釋放素、綠膿桿菌、公豬。

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