A single dose of a commercial anti-gonadotropin releasing factor vaccine has no effect on testicular development, libido, or sperm characteristics in young boars

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Summary

Objectives: To evaluate the effects of one dose of an anti-gonadotropin releasing factor (GnRF) vaccine on testicular development, sexual behavior, and sperm characteristics in young boars.

Materials and methods: A total of 48 pigs were equally allocated to two treatments, Controls and Immunized, with a single dose of an anti-GnRF vaccine at 16 weeks of age. Sexual behavior was evaluated 5 to 8 weeks later. Of these 48 pigs, 22 (12 Controls, 10 Immunized) underwent weekly semen collections for 14 consecutive weeks, starting 17 weeks after immunization. One week after completion of the weekly collections, six boars per treatment underwent daily collections for 7 days. Blood for testosterone analysis was collected from seven animals per group at 0, 2, 4, 8, 12, 16, 20, 24, and 28 weeks post immunization.

Results: There were no statistical differences between treatments in gonad size, the sexual behavior test, qualitative and quantitative semen characteristics, sperm morphology, time to mount, ejaculation time, or serum testosterone concentrations. There was no histological evidence of an alteration in onset and development of puberty in the immunized pigs.

Implications: Under the conditions of this study, one dose of an anti-GnRF vaccine given to 16-week-old boars has no effect on testicular development, sexual behavior, or sperm characteristics. As final replacement-boar testing is typically conducted after 24 weeks of age, a priming dose of vaccine could be given prior to boars undergoing final testing without negative impact on testicular development and future breeding potential.

Resumen - Una dosis única de la vacuna comercial del factor de liberación de anti-gonadotropina no tiene efecto en el desarrollo testicular, libido, o las características del esperma en machos jóvenes

Objetivos: Evaluar los efectos de una dosis de la vacuna de factor de liberación de antigonadotropina (GnRF) en el desarrollo testicular, conducta sexual, y características de esperma en machos jóvenes.

Materiales y métodos: Se distribuyó equitativamente un total de 48 cerdos a dos tratamientos, Controles e Inmunizados, con una dosis única de la vacuna anti-GnRF en la 16 semanas de edad. Se evaluó la conducta sexual 5 a 8 semanas posteriores. De estos 48 cerdos, 22 (12 Controles, 10 Inmunizados) fueron sometidos a colecciones de semen semanales por 14 semanas consecutivas, empezando 17 semanas después de la inmunización. Una semana después de finalizar las colecciones semanales, seis machos por tratamiento fueron sometidos a colecciones diarias durante 7 días. Se colectó sangre para análisis de testosterona de siete animales por grupo la semana 0, 2, 4, 8, 12, 16, 20, 24, y 28 post inmunización.

Resultados: No hubo diferencias estadísticas entre los tratamientos en tamaño de gónada, la prueba de conducta sexual, características cuantitativas y cualitativas de semen, morfología del esperma, tiempo para montar, tiempo de eyaculación, o concentraciones de testosterona en el suero. No hubo evidencia histológica de una alteración en el inicio y desarrollo de la pubertad en los cerdos inmunizados.

Implicaciones: Bajo las condiciones de este estudio, una dosis de una vacuna anti-GnRF aplicada a machos de 16 semanas de edad no tiene efecto en el desarrollo testicular, conducta sexual, o características del esperma. Como la prueba final al macho de reemplazo se efectúa típicamente después de la semana 24 de edad, se podría aplicar una dosis de vacuna preparatoria antes de que los machos sean sometidos a la prueba final sin un impacto negativo en el desarrollo testicular y su futuro potencial de cría.

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Résumé - Une dose unique d'un vaccin commercial anti-facteur relâchant de gonadotropine n'a aucun effet sur le développement testiculaire, la libido, ou les caractéristiques du sperme de jeunes verrats

Objectifs: Évaluer les effets d'une dose de vaccin anti-facteur relâchant de la gonadotropine (GnRF) sur le développement testiculaire, le comportement sexuel, et les caractéristiques du sperme de jeunes verrats.

Matériels et méthodes: Un total de 48 porcs ont été distribués également à deux traitements, Témoins et Immunisés, avec une dose unique d'un vaccin anti-GnRF à 16 semaines d'âge. Le comportement sexuel fut évalué 5 à 8 semaines plus tard. De ces 48 porcs, 22 (12 Témoins, 10 Immunisés) furent soumis à

une collecte hebdomadaire de semence pendant 14 semaines consécutives, débutant 17 semaines après l'immunisation. Une semaine après avoir terminé les collectes hebdomadaires, six verrats par groupe de traitement furent soumis à une collecte quotidienne pendant 7 jours. Du sang pour dosage de la testostérone fut prélevé de sept animaux par groupe à 0, 2, 4, 8, 12, 16, 20, 24, et 28 semaines post-immunisation.

Résultats: Il n'y avait aucune différence significative entre les groupes de traitement en ce qui a trait à la taille des gonades, le test de comportement sexuel, les caractéristiques qualitatives et quantitatives de la semence, la morphologie du sperme, le temps de la monte, le temps d'éjaculation, ou les concen-

trations de testostérone sérique. Il n'y avait pas d'évidence histologique d'une modification dans le début et le développement de la puberté chez les porcs immunisés.

Implications: Dans les conditions de la présente étude, une dose d'un vaccin anti-GnRF donné à des verrats âgés de 16 semaines n'avait aucun effet sur le développement testiculaire, le comportement sexuel, ou les caractéristiques du sperme. Étant donné que les épreuves finales sur les verrats de remplacement sont effectuées après 24 semaines d'âge, une dose d'amorce de vaccin pourrait être donnée préalablement aux épreuves finales sur les verrats sans impact négatif sur le développement testiculaire et le potentiel reproducteur futur.

Immunization against gonadotrophin releasing factor (GnRF), producing an "immunological" castration, is an increasingly used alternative to physical castration of young piglets, controlling boar taint while maintaining most of the production efficiencies associated with entire males.[1] While this process uses the immune system to stimulate production of specific anti-GnRF antibodies, it is not classified as a "vaccine" in some countries and is thus frequently referred to as an "immunological product." The mode of action is nevertheless that of a classical vaccine, and in this report the term vaccine is used for simplicity. A commercial anti-GnRF vaccine is now available in many countries from the same manufacturer under several trade names (Improvac, Improvest, Vivax, Innosure), hereafter referred to as Improvac (Zoetis, Florham Park, New Jersey). The immunizing antigen in Improvac comprises a synthetic analogue of endogenous mammalian GnRF conjugated to a carrier protein. The result is a large protein molecule that has no intrinsic hormonal activity and is foreign to the immune system.[2] Formulated with an appropriate adjuvant, it stimulates the immune system, after two doses, to transiently produce high concentrations of circulating antibodies that can bind and inhibit the action of natural GnRF.[1]

To achieve effective suppression of testes function and clearance of boar taint, two doses must be given at least 4 weeks apart.[1,3-5] The first dose serves to prime the immune system and results in only a small increase in detectable circulating

anti-GnRF antibodies[3-5] with, at the time of the second dose, no detectable effect on circulating testosterone concentrations[1,3-5] or testes growth.[1] After the second dose, there is a strong antibody response that results in temporary suppression of testicular function.[1,3-5]

The procedure may be used on male pigs that fail the selection procedure for breeding boars, thus controlling boar taint and allowing their use for human consumption. However, the minimum time from first dose to slaughter is typically 8 weeks; thus, rejected breeding boars would need to be retained for at least 8 weeks after rejection (ie, after the first immunization) before they could be sold free of boar taint. Giving the first dose prior to selection testing would allow the second dose to be given at the time of the rejection decision, thus potentially saving 4 or more weeks. While the available data suggest that the first dose has no or minimal physiological effect,[1,3-6] and thus no impact on subsequent breeding performance, specific detailed studies are lacking. The objective of the present study was to test, for the first time to the authors' knowledge, whether a single priming dose of the anti-GnRF vaccine, Improvac, has effects on testicular development, libido, or sperm characteristics in young boars.

Materials and methods

This study and all procedures used in the study were conducted in compliance with the Brazilian regulatory guidelines for the ethical use of animals and animal welfare.[7]

Animals, housing, and feeding

Forty-eight healthy entire male pigs of a commercial Landrace × Large White genotype were selected for the trial at 14 weeks of age from a commercial swine farm in Brazil and transferred to the research facility. All animals were born in the same week, weaned on the same day, and identified with individually numbered ear tags. From 15 weeks of age, the pigs were housed in individual pens with a minimum space of 8.8 m². Floors were partially slatted and the barn sides were open with closable curtains. Between 15 and 20 weeks of age, the animals were fed ad libitum with a commercial grower-finisher ration (crude protein 16%). From 21 weeks of age (5 weeks after immunization), the animals received 2.0 to 2.5 kg per day of a commercial boar ration (crude protein 15%), fed once a day. Body condition was evaluated each time the pigs were weighed, and the amount of feed adjusted when necessary. The animals had free access to water via nipple drinkers.

Experimental design

At 16 weeks of age, using randomly generated numbers, the pigs were allocated into two equal groups: 24 Controls and 24 Immunized. The individual animal represented the experimental unit. At 16 weeks of age, the Control group were injected subcutaneously behind the left ear with 2 mL of non-pyrogenic sterile saline, and the Immunized group were similarly injected with 2 mL of the anti-GnRF vaccine (Improvac batch 009/08; Zoetis, Florham Park, New Jersey). The batch of Improvac

used was a full commercial batch produced under Good Manufacturing Practice (GMP) conditions, which require that every batch released must pass a full quality assessment, including potency. Personnel who were not directly involved in the collection of experimental data delivered treatments, while personnel designated to carry out the experimental observations and data collection were blind to treatment during the experimental period.

Because of space and labor restrictions and the fact that this study had multiple components, some of which were mutually incompatible, the 24 available animals per treatment were randomly shared among the components. A schedule of events for the study is shown in Figure 1. At the start of the trial, a total of 14 animals (seven per treatment) were selected by random number generation for assessment of serum testosterone at various time points during the study. Blood was collected from these animals at the following time points: treatment day (16 weeks of age), 2 and 4 weeks after treatment, and then every 4 weeks until 44 weeks of age. The same animals were used in all collections, and these animals were not used for histological assessment of the testes structure. Approximately 10 mL of blood was collected by jugular venipuncture on each occasion. Serum was obtained and stored at -20°C until used for testosterone analysis. Also at the start of the study, 10 animals per treatment were randomly allocated to be castrated, under general anesthesia, to enable the testes and epididymides to be weighed and the testes to be assessed histologically. Three animals per treatment were castrated at each of 4 and 13 weeks post treatment, and a further four per treatment were castrated at 31 weeks post treatment. All 24 pigs per treatment were individually weighed at selection (14 weeks of age) and at immunization (16 weeks of age). All available pigs from the initial 24 per treatment were again weighed 4, 13, 24, and 31 weeks after immunization. At each of these times, the width of the scrotum at its maximum width was also measured with a pair of engineering calipers.

Testosterone analysis

Quantification of testosterone was performed using liquid chromatography with tandem mass spectrometric detection. This method was chosen instead of one of the many radioimmunoassay kits available because numerous studies have proven the liquid chromatography-mass spectrometric (LC-MS) method to be a more reliable method of determining serum testosterone concentration.[8,9] The LC-MS method has been validated for pig serum consistent with the current Federal Drug Administration (FDA) Guidelines for Bioanalytical Methods Validation.[9] Briefly, testosterone was extracted from porcine serum using a 96-well automated liquid-to-liquid extraction at alkaline pH with ethyl acetate. Before extraction, isotope-labeled testosterone was added as an internal standard (testosterone-d3). The organic layer was collected, transferred to a new 96-well plate, and evaporated to dryness. The residue was reconstituted with a 55% acetonitrile, 45% purified water, 0.1% formic acid mobile phase and injected into a liquid chromatography-mass spectrometer using a Betasil C18 column (Keystone Scientific Inc, Bellefonte, Pennsylvania). The lower and upper limits of quantification were 0.35 and 69.4 nmol per L, respectively. Testosterone-free porcine serum samples collected from castrated boars were used as "blank" reference samples and stored under the same conditions as the study samples. Each assay included blank biological matrix and a series of six pre-prepared calibration standards, together with three in-house prepared quality control (QC) samples within the assay's range of quantification. The mean intra- and inter-assay bias of the QC samples was < 15.0%, which is consistent with FDA guidelines.[10]

Evaluation of sexual behavior

Sexual-behavior evaluations were performed when the animals were 21 to 24 weeks of age (5 and 8 weeks after treatment) by exposure of the boars to estrous gilts. Ten gilts, approximately 5 to 6 months of age, were housed as a group in the same building as the males. Thirty-eight boars (19 animals per treatment) underwent sexual-behavior evaluation. Over a 15-minute period, each boar was individually exposed to an estrous female of compatible body size in a 2 × 3-m area. The following parameters were recorded: number of pigs mounting on the first presentation to an estrous female, number of incorrect mounting attempts until a correct mount was made, and time from entering the pen until a correct mount was made. The test was concluded when the male correctly mounted. Intromission and mating were not allowed.

Semen collection and evaluation

After the sexual-behavior evaluation was concluded, 22 boars (12 Controls and 10 Immunized) were trained for semen collection on a fixed dummy using the gloved-hand method.[11] The boar was considered trained when it performed the mount within 10 minutes after being presented to the dummy and allowed the total ejaculate to be collected. Over 14 consecutive weeks, from week 17 after treatment (33 weeks of age) onwards, the trained boars were subjected to semen collection at 7-day intervals. One week after completion of the 14 weekly collections, six boars per treatment underwent an intensive daily semen collection for 7 days. The same person performed all semen collections. The times between entering the collection room and an effective mount on the dummy and the ejaculation time were recorded. The quantitative and qualitative characteristics of the ejaculate were evaluated for all collections. The liquid and gel fractions of the ejaculate were separated at the time of collection using a filter adapted to the collection vial. The liquid volumes, weight of the gel fraction, sperm motility, sperm concentration, total number of spermatozoa, and sperm morphology were recorded. Sperm morphology was evaluated in all ejaculates collected during the weekly collection phase, and from ejaculates collected on days 1, 4, and 7 of the daily collection phase. The same person performed all semen examinations.

Volume of the liquid fraction was determined by weighing the liquid fraction of the ejaculate and calculating the volume, assuming a semen density of 1 g per mL. Sperm motility was determined by clear field microscopy at 100× magnification, evaluating the percentage of motile spermatozoa in a semen drop between a glass slide and cover slip previously heated to 37°C. Five to six fields per slide were evaluated. Motility evaluation was repeated three times for each ejaculate, and the best result obtained was then recorded.

Sperm concentration was determined in a hemocytometer chamber (Neubauer) and the result expressed in number of cells per mm^3 semen. Total number of cells in the ejaculate was obtained by multiplying the sperm concentration by the ejaculate volume. Sperm morphology was examined by phase contrast microscopy at 1000× magnification in a wet preparation, with semen fixed in formol citrate solution. The semen samples were prepared immediately after ejaculate collection and examined on the same day. In each ejaculate, 200 cells were

Figure 1: Timeline for the 31-week duration of a study to evaluate the effects of one dose of an anti-gonadotropin releasing factor vaccine on testicular development, sexual behavior (19 boars/treatment), and sperm characteristics in young boars from a commercial facility. At 16 weeks of age, the Control group (n = 24) were injected subcutaneously behind the left ear with a single 2-mL dose of non-pyrogenic sterile saline, and the Immunized group (n = 24) were similarly injected with 2 mL of anti-GnRF vaccine (Improvac; Zoetis, Florham Park, New Jersey). Shading indicates the assessments performed each study week. Individual body weight and scrotal width measurements were made on all available animals at each time point. Blood samples were collected from seven pre-selected pigs per treatment. Semen was evaluated on 12 Control and 10 Immunized pigs at each time point indicated, with the exception of the seven daily collections in study week 31 when six pigs per treatment were assessed. Three pigs (*) or four pigs (†) per treatment were designated for castration for histological assessment of the testes.

Study week	Age (weeks)	Weighed	Scrotal width measurement	Blood sample	Castration	Semen evaluation	Sexual behavior
0	16	Yes	Yes	Yes	No	No	No
2	18	No	No	Yes	No	No	No
4	20	Yes	Yes	Yes	Yes*	No	No
5	21	No	No	No	No	No	Yes
7	23	No	No	No	No	No	No
8	24	No	No	Yes	No	No	Yes
12	28	No	No	Yes	No	No	No
13	29	Yes	Yes	No	Yes*	No	No
16	32	No	No	Yes	No	No	No
17	33	No	No	No	No	Yes	No
18	34	No	No	No	No	Yes	No
19	35	No	No	No	No	Yes	No
20	36	No	No	Yes	No	Yes	No
21	37	No	No	No	No	Yes	No
22	38	No	No	No	No	Yes	No
23	39	No	No	No	No	Yes	No
24	40	Yes	Yes	Yes	No	Yes	No
25	41	No	No	No	No	Yes	No
26	42	No	No	No	No	Yes	No
27	43	No	No	No	No	Yes	No
28	44	No	No	Yes	No	Yes	No
29	45	No	No	No	No	Yes	No
30	46	No	No	No	No	Yes	No
31	47	Yes	Yes	No	Yes†	Yes (daily)	No

evaluated. Alterations in sperm morphology were classified according to the spermatozoa anatomic site where they occurred. Primary and secondary acrosome defects, abnormal head and neck, mid-piece defects, and abnormal tails, as well as proximal and distal droplets, were recorded.

Histological evaluation

The pigs selected for castration were sedated with acepromazine at 0.2 mg per kg body-weight intramuscularly (Lab Vetnil, Sao Paulo, Brazil) and anesthetized with Zoletil 50 (a combination of tiletamine hydrochloride and zolazepam hydrochloride; Virbac, Carros Cedex, France) at 3 to 5 mg per kg body weight intramuscularly. The testicles were separated from the epididymides immediately after castration, and the testes and epididymides were weighed separately. Samples (approximately 2 × 2 × 1 cm) were collected from the capitata extremity, the mid-third, and the caudal extremity of the left testicle of each animal and immediately fixed in Bouin's solution for 24 hours. The samples were embedded in paraffin, and 6-μ sections were cut and stained with hematoxylin and eosin. Each section was examined at 100× power to visually assess seminiferous tubule size, degree of luminal formation, type of sperm cells present, and phase of testicular development.

Data analysis

Data was analyzed using the SAS statistical package release 9.1.3 (SAS Institute Inc, Cary, North Carolina). A one-way ANOVA F-test was used to determine if there was an initial body weight difference between

the two treatments when the pigs were weighed at 3 to 4 weeks of age. Analysis of covariance was used to test for differences in body weight at 26, 90, 168, and 214 days of age, using the initial weight as covariable. Testosterone data were analyzed on log-transformed data by ANOVA F-test with interaction between treatment and time. Treatment and time were considered fixed effects, with animals as a random effect. Pairwise comparisons with time were tested using the Tukey-Kramer test. Scrotal width, testes weight, and epididymides weight were also analyzed using an ANOVA F-test, with pairwise comparisons using the Bonferroni test. The chi-square test and Fisher's exact test were used to analyze the sexual-behavior information using categories of one or two presentations: zero and at least one for the number of mounts, and 1 minute and ≥ 2 minutes for the time to mount. Except for motility, a repeated measures ANOVA F-test was used for semen data with interaction between treatment and time. Treatment and time were considered fixed effects with animals as a random effect. Because there was no variability in the motility data, an ANOVA test was not used and only descriptive statistics were used for motility. For all tests, $P < .05$ was considered statistically significant. The histological assessments were qualitative only, and no statistical evaluations were performed on the histological observations.

Results

There were no statistically significant differences between treatments for any of the testicular, sexual-behavior, or ejaculate parameters. Body weight did not differ between treatment groups at the start of the study (16 weeks of age): 61.9 kg versus 61.5 kg for Controls and Immunized, respectively. Body weights of the Control and Immunized groups did not differ at the other time points in the study: 83.9 kg versus 84.3 kg, 133.9 kg versus 133.9 kg, 181.7 kg versus 183.6 kg, and 198.4 versus 202.0 kg for Controls and Immunized, respectively, at 4, 13, 24, and 31 weeks after immunization (20, 29, 40, and 47 weeks of age).

In situ maximum scrotal width along with the weight of the testes and epididymides did not differ between treatments at all time points assessed and are presented in Table 1. The only differences in scrotal width, testes, or epididymides weight were the increase in these parameters with time as the pigs in both groups matured. As expected for a hormone

that is secreted episodically, the testosterone concentrations were highly variable across time, but did not differ ($P > .05$) between treatments at any time point (Table 2).

There were no statistically significant differences between treatments for any sexual-behavior parameter. All pigs mounted the estrous female when presented, with 15 of the 19 Control pigs (79%) and 19 of the 19 Immunized pigs (100%) mounting on their first presentation ($P = .11$). The number of incorrect mounts before a correct mount was achieved also did not differ between treatments, with 12 of the 19 Control pigs (63%) and eight of the 19 Immunized pigs (42%) mounting on the first attempt ($P = .33$). Eight of the 19 Control pigs (47.0%) pigs and seven of the 19 Immunized pigs (37%) mounted within the first minute of exposure to an estrus female ($P = .74$). The maximum time to perform a correct mount was 7 minutes, and no pigs exceeded four incorrect mounting attempts. For the weekly semen collections, there were no significant differences between the groups in the quantitative or qualitative characteristics of semen (Table 3). All values were within expected ranges. No parameter in either treatment showed any deterioration over the course of the 14 weeks of weekly collections.

During the week when semen was collected daily, there was a gradual deterioration in many of the parameters assessed. However, the time-dependent decline in parameter values occurred equally across both treatments (Table 4). The deterioration in gel weight, liquid fraction volume, sperm concentration, total number of sperm per ejaculate, and number of abnormal cells was statistically significant in both treatments ($P < .05$) when the results of the first and last daily collections were compared. There were no between-treatment statistical differences for any parameter during the period of daily collections.

Visually, there was no qualitative histological evidence of any alteration in the onset and development of puberty in the Immunized group compared to the Controls. Qualitative histological examination revealed normal development of the seminiferous parenchyma and support structures, including Leydig cells and seminiferous tubules, in the two treatments. Tubule transformation and spermatogenesis did not differ visually between the two groups. In the youngest pigs castrated (4 weeks after treatment;

20 weeks of age), testicular development in both groups was in the pre-pubertal stage (luminal formation, incipient spermatogenesis with presence of spermatids). By 29 weeks of age (13 weeks after treatment), both groups had evolved to the pubertal stage (complete spermatogenesis and spermatozoa in the lumen of the tubules, complete luminal formation). By 47 weeks of age (31 weeks after treatment), the two groups were in the post-pubertal stage (increased numbers of spermatocytes and spermatids, adequate yield of spermatogenesis). The presence, structure, and number of Leydig cells did not differ morphologically between the treatment groups at the three ages.

Discussion

In countries where immunization against GnRF has been used commercially, there have been many expressions of interest to use the procedure on breeder boars that fail the selection process. This would allow such animals to be sold free of boar taint and allow their use for human consumption. However, the minimum time from first dose to slaughter is typically 8 weeks, thus reject breeding boars would have to be kept for at least 8 weeks after rejection before they could be sold free of boar taint. If the first dose could be given prior to final selection testing, the second dose could be given at the time of the rejection decision, potentially saving 4 or more weeks. While the available data suggest that the first dose has minimal physiological effects on testes function,[1,3-5] and should have no impact on subsequent breeding performance, specific detailed studies were lacking. Such information arises from very limited observations made at the time the second dose is given, generally 4 to 6 weeks after the first dose, at 18 to 22 weeks of age.[1,3]

The results of the present study demonstrate that a single dose of Improvac given at approximately 16 weeks of age has no detectable effect on testes development, sexual behavior, or sperm characteristics of young boars. Giving the initial dose earlier than at 16 weeks of age (for example, at 10 to 12 weeks of age) is unlikely to alter the outcomes of the current experiment. Research data on file with Zoetis shows that there was no effect of an initial dose given at 4 weeks of age on testosterone concentrations or testes width (growth) at 19 weeks of age, when the second dose was administered (written communication; Professor Frank

Table 1: Mean (standard deviation) in situ scrotal width (cm) at maximum width and, for the subsets of castrated pigs, trimmed testes and epididymides weights (g) in Controls and Immunized pigs*

Week of study	Age of pigs (weeks)	Scrotal width (cm) Control	Scrotal width (cm) Immunized	Testes weight (g) Control	Testes weight (g) Immunized	Epididymides weight (g) Control	Epididymides weight (g) Immunized
0	16	7.79 (0.67)	7.67 (0.64)	ND	ND	ND	ND
4	20†	10.12 (0.97)	9.97 (0.64)	246.86 (84.21)†	265.94 (26.07)†	57.28 (7.39)†	57.65 (6.88)†
13	29†	14.12 (0.68)	14.03 (0.84)	628.33 (73.36)†	571.67 (65.65)†	135.67 (11.93)†	140.67 (14.74)†
24	40	15.29 (0.97)	14.96 (0.97)	ND	ND	ND	ND
31	47‡	15.68 (0.97)	15.84 (1.43)	782.00 (89.60)‡	690.25 (121.05)‡	222.50 (21.81)‡	197.75 (19.57)‡

* Study and pigs described in Figure 1. Scrotal width measurements were made on 24 pigs per treatment at 16 and 20 weeks of age, 21 per treatment at 29 weeks of age, and 18 per treatment at 40 and 47 weeks of age. There were no statistical differences between treatments ($P > .05$) for any parameter at any time point (ANOVA F-test; pairwise comparisons tested with the Bonferroni test).

† Three pigs per treatment castrated.

‡ Four pigs per treatment castrated.

ND = not determined: no pigs castrated.

Table 2: Mean (standard deviation) serum testosterone concentration at various time points in seven Control pigs and seven Immunized pigs*

Week of study	Age of pigs (weeks)	Serum testosterone concentration nmol/L Control	Serum testosterone concentration nmol/L Immunized
0	16	19.6 (14.4)	14.7 (10.7)
2	18	8.4 (3.3)	4.5 (4.0)
4	20	16.6 (15.6)	4.6 (2.9)
8	24	5.4 (2.2)	4.5 (7.0)
12	28	13.5 (5.7)	17.6 (12.8)
16	32	18.9 (9.5)	24.7 (15.7)
20	36	4.1 (3.3)	5.2 (2.8)
24	40	11.2 (5.0)	19.2 (12.0)
28	44	3.0 (1.4)	4.3 (4.9)
Overall mean		11.3 (10.0)	11.5 (11.4)

* Pigs and study described in Figure 1. There were no statistical differences ($P > .05$) between treatments for testosterone concentration at any time point (ANOVA F-test; pairwise comparisons tested with the Tukey-Kramer test).

Dunshea, Chair of Agriculture, University of Melbourne, Melbourne, Victoria, Australia; 1996).

A limitation of this study is that a positive control (ie, receiving two full doses of Improvac) was not included. Thus, it is legitimate to ask whether the specific batch of Improvac was indeed biologically active and whether the immune system was actually primed. The batch of Improvac used was a full commercial batch produced under GMP conditions, which require that every batch released must pass a full quality assessment, including potency. In addition, this batch performed satisfactorily in the field in Brazil (e-mail communication; Dr Fabio Teixeira, Technical Manager, Zoetis, Brazil), where on-line assessment of reduced testes size is routinely performed in all abattoirs. There were no reports of a lack of efficacy from these plants during the time that this batch was used in the field. Thus it is reasonable to conclude that the one dose used in this study would have in fact adequately primed the immune system with no detectable deleterious effects on testes development or sexual behavior.

There is a wealth of data available on the effects of immunization with two doses of Improvac on growth performance, boar taint, testes function, and sexual behavior. Several reports have demonstrated that immunization with two doses is efficient in eliminating boar taint and, compared with physical castrates, results in better growth performance and carcass characteristics.[1,12-15] In addition, as the immunized boar's testes function is temporarily inhibited, concentrations of testosterone and other sexual hormones are suppressed. As a consequence, concentrations of androstenone and skatole in the subcutaneous fat are suppressed.[1,4,6,16] Consistent with a suppression of testes function, other studies have shown that the animals' sexual and aggressive behavior is suppressed compared to that in physical castrates.[4,17,18]

Administration of the first dose of Improvac at approximately 16 weeks of age coincides with development of puberty in boars at 13 to 20 weeks of age.[19,20] This period is characterized by rapid growth of the testes and by proliferation of Sertoli cells and establishment of spermatogenesis, which leads to the presence of free sperm in the lumina of the seminiferous tubules. Follicle stimulating hormone, luteinizing hormone, and testosterone all play fundamental roles in regulating this process. Serum testosterone concentrations reach a maximum during puberty, followed by a slight decrease before stabilizing.[21] Full sexual maturity generally takes place at approximately 8 months of age.

In the present study, various measures of testes function, testes morphology, and semen characteristics were made from 16 weeks of age (the middle of the pubertal period when the first dose of Improvac was administered) until 47 weeks of age. Thus, it was possible to monitor the effects of a single dose on development of the testes during the transient stage of puberty until the post-pubertal stage when sexual and reproductive maturity were reached. The results obtained indicate that, under these experimental conditions, administration of a single dose of Improvac did not affect testes development or function. At all ages that were evaluated, the size of the gonads, both the in situ measurements and the weight of testes and epididymides, did not differ ($P > .05$) between the Immunized pigs and the Controls that received the placebo.

The growth of testes and epididymides was more intense in early puberty, between 16 and 20 weeks of age, as previously described in swine.[19] In boars 29 and 47 weeks of age, the mean weights of the testes (pair) in the control and treated groups were not statistically different and were similar to those observed by Silva.[19] The administration of a single dose of Improvac did not influence development and maturation of the testes structure, as determined by morphology and morphometry observations of the testes. Performance in the sexual behavior tests did not differ between males in the two treatment groups. The sexual-behavior observations reported here are consistent with those reported by Ferreira et al.[22]

In this study, semen collection was started at 33 weeks of age, coinciding with a common

age at which semen donors are introduced to regular semen collections in commercial artificial insemination centers.[23] There was no difference in the results of quantitative and qualitative characteristics of semen between the treatment groups: all parameters were within the physiological standards of sperm production for swine.[21] Finally, there was no difference in the serum testosterone concentrations of vaccinated and non-vaccinated pigs, indicating that a single dose of Improvac did not influence the serum profile of this hormone. A large variation in the testosterone concentration was observed throughout the study, which can be attributed to the episodic characteristic of testosterone release.

The detailed findings on testicular development, sexual behavior, and semen

Table 3: Mean (standard deviation) across the 14 weeks of weekly semen collections for measures of sexual behavior and semen assessment in 12 Control pigs and 10 Immunized pigs*

	Control	Immunized
Time to mount (min)	3.44 (2.89)	2.61 (1.81)
Ejaculation time (min)	6.74 (2.35)	6.64 (2.26)
Gel weight (g)	49.2 (22.1)	56.2 (24.6)
Liquid fraction of ejaculate (mL)	233.5 (82.0)	257.0 (77.3)
Sperm concentration (10^6/mm^3)	0.28 (0.06)	0.26 (0.03)
Total number cells (× 10^9)	63.1 (21.0)	66.8 (19.8)
Abnormal cells (%)	5.56 (2.70)	7.51 (5.08)
Motility (%)	81.1 (5.8)	77.5 (8.7)

* Pigs and study described in Figure 1. With the exception of sperm motility, there were no statistical differences between treatments ($P > .05$) in any parameter (repeated-measures ANOVA F-test). As there was no variability in the motility data, only descriptive statistics were used for this parameter.

Table 4: Mean (standard deviation) across the 7 days of daily semen collections and on the first and last days of the week of daily collections for measures of sexual behavior and semen assessment in six Control pigs and six Immunized pigs*

	Mean for 7 days		Day 1 mean		Day 7 mean	
	Control	Immunized	Control	Immunized	Control	Immunized
Time to mount (min)	2.88 (1.97)	3.50 (2.98)	2.50 (1.97)	3.33 (3.01)	3.33 (0.52)	3.33 (0.82)
Ejaculation time (min)	6.36 (1.91)	6.48 (2.67)	7.50 (1.64)	7.67 (4.18)	6.67 (1.03)	4.50 (1.05)
Gel weight (g)	45.26 (21.5)	44.8 (29.4)	52.33 (9.58)	71.00 (52.49)	28.50 (11.41)	25.50 (9.93)
Liquid fraction of ejaculate (mL)	215.9 (97.9)	193.1 (84.0)	282.83 (62.60)	299.33 (129.23)	135.83 (60.38)	115.33 (32.32)
Sperm concentration (10^6/mm^3)	0.22 (0.07)	0.21 (0.06)	0.318 (0.06)	0.313 (0.07)	0.156 (0.02)	0.159 (0.02)
Total number cells (× 10^9)	49.0 (32.2)	43.2 (25.8)	90.46 (30.18)	87.75 (24.97)	20.24 (6.33)	18.73 (7.28)
Abnormal cells (%)	10.58 (6.68)	15.17 (10.00)	5.00 (1.87)	9.75 (9.22)	17.58 (6.10)	21.58 (7.49)

* Pigs and study described in Figure 1. There were no statistical differences between treatments ($P > .05$) in any parameter (repeated-measures ANOVA F-test).

characteristics, reported here for the first time (to the knowledge of the authors), are to be expected and are fully consistent with the limited reports of similar testes function at the time of the second dose of the vaccine.[1,3-5] Because the first dose serves only to prime the immune system, possible replacement breeding boars could be given a priming dose of Improvac with confidence that there will be no deleterious short- or long-term effects on breeding performance.

Implications

- Under the conditions of this study, administration of a single dose of the anti-GnRF vaccine, Improvac, to intact male pigs at 16 weeks of age has no effect on testicular development, sexual behavior, or sperm characteristics over the subsequent 32 weeks (to 48 weeks of age).

- As final boar testing in replacement studs is typically conducted after 24 weeks of age, a priming dose of Improvac could be given to boars at approximately 16 weeks of age, prior to undergoing final testing, without any negative impact on testicular development and future breeding potential.

- Boars that subsequently fail the selection test could then be given a second dose of Improvac, enabling them to be slaughtered free of boar taint approximately 4 weeks later.

Acknowledgement:

Conflict of interest

Drs Oliveira, Mathur, and Allison are all employees of Zoetis. Dr Scheid is the head of Scheid Assessoria Agropecuária, the Brazilian company that conducted the research under contract with Zoetis. Dr Soncini is an employee of Scheid Assessoria Agropecuária. Dr Hennessy, an honorary Senior Fellow at the University of Melbourne, was provided a consultancy fee to review the research report from Scheid Assessoria Agropecuária and prepare this paper for publication.

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