

Efficiency of the anti-GnRH vaccine for castration of lambs intended for finishing in pastures

Eficiência da vacina anti-GnRH para castração de cordeiros destinados à terminação a pasto

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Highlights

Castrated animals are earlier for deposition of fat in the carcass.
Surgical castration - sterilization method that causes postoperative complications.
Anti-GnRH vaccine works as a suppressor of the gonadotropin-releasing hormone.
The effect of the anti-GnRH vaccine on lamb semen parameters was evaluated.
The vaccine promoted infertility in animals.

Abstract

It was aimed to evaluate anti-GnRH vaccine efficiency on productive characteristics, seminal performance, size, and histology of testicles of lambs submitted to immunocastration. Twenty contemporary Texel lambs were evaluated, being 10 animals received two doses of 1 mL of anti-GnRH vaccine and 10 received two doses of 1 mL of saline, with an interval of 30 days. Seminal characteristics, weight and histological cut for the testicles, productive performance, and blood parameters were analyzed. Andrological monitoring was carried out every 30 days. All animals were kept on pasture of *Urochloa* spp. receiving daily supplementation protein-energetic by 90 days. There was effect of immunocastration on slaughter weight (44.3 vs. 48.3 kg), total gain (9.9 vs. 10.3 kg), daily gain (104.5 vs. 108.9 g/day), and feed conversion (5.83 vs. 5.97) respectively to immunocastrated and intact lambs. There was immunocastration effect on testicle weight (0.09 vs. 0.35 g), motility (4.5 vs. 61.0%), vigor (0.40 vs. 3.00), volume (0.09 vs. 0.74 mL) and swirling (0.20 vs. 2.70) in the

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third semen collection. Immunocastration through two doses of 1 mL of anti-GnRH vaccine with a 30-day interval between doses, causes infertility in sheep and can be adopted for lambs.

Key words: *Haemonchus*. Selective control. Sensitivity. Small ruminants. Specificity.

Resumo

O objetivo foi avaliar a eficiência da vacina anti-GnRH quanto às características de desempenho produtivo e seminais, tamanho e histologia dos testículos de cordeiros submetidos à imunocastração. Foram avaliados 20 cordeiros contemporâneos cruzados SRD x Texel, dos quais 10 animais receberam duas doses de 1 mL de vacina anti-GnRH e 10 receberam duas doses de 1 mL de soro fisiológico, com intervalo de 30 dias entre doses. As variáveis analisadas foram concentração espermática, motilidade, vigor, turbilhonamento e volume de ejaculado para o sêmen, peso e corte histológico para os testículos, peso ao abate, ganho de peso total, ganho médio diário, consumo médio de suplemento e conversão alimentar para o desempenho produtivo e GGT, AST, albumina, creatinina, proteínas totais e ureia para o sangue. A cada 30 dias foi realizado o acompanhamento andrológico para verificação do efeito da vacina sobre características seminais e testiculares dos animais. Durante os 90 dias de duração do experimento, os animais foram mantidos a pasto de *Urochloa* spp., com suplementação diária, sendo abatidos ao final deste período. Houve diferença entre animais imunocastrados e inteiros respectivamente, para o peso ao abate (44,3 vs. 48,3 kg), ganho de peso total (9,9 vs 10,3 kg), ganho médio diário (104,5 vs. 108,9 g/dia) e conversão alimentar (5,83 vs. 5,97). Houve efeito significativo para motilidade (4,5 vs. 61,0%), vigor (0,40 vs. 3,00), volume (0,09 vs. 0,74 mL) e turbilhonamento (0,20 vs. 2,70) na terceira colheita de sêmen, que foi 30 dias após a segunda dose da vacina. Houve efeito significativo para peso dos testículos (0,09 vs. 0,35 g). No perfil bioquímico do sangue dos animais não houve alterações significativas durante o experimento em ambos os tratamentos. A imunocastração através de duas doses de 1 mL de vacina anti-GnRH com 30 dias de intervalo entre as doses, causa infertilidade em ovinos e pode ser adotada sem prejudicar o desempenho e a saúde de cordeiros terminados a pasto.

Palavras-chave: Avaliação andrológica. Características seminais. Imunocastração. Ovinos.

Introduction

Surgical castration is performed in order to reduce the anabolic effect of testosterone and increase the deposition of fat in the carcass, in addition to decreasing the male behavior promoted by this hormone, reducing physical activity and energy expenditure in fights for dominance and sodomy (Machado et al., 2018). However, the surgical castration technique is a bloody sterilization method, which does not value the precepts of animal well-being, in addition to

causing several postoperative complications, such as inflammation and myiasis in the wound, even causing death, resulting in death. Expenditure on medicines and economic losses (Price, Adams, Huxsoll, & Borgwardt, 2003).

The change in the behavior of castrated males also causes greater docility for handling and the reduction of disputes between animals, leading to a lower number of carcass injuries and, consequently, problems in meat quality (Amatayakul-Chantler et al., 2013).

In addition to carcass damage, the off flavor or unpleasant taste of sheep meat, which, although not yet fully understood, leads to the formation of volatile compounds responsible for changes in meat flavor and aroma, and in some cases, are associated with changes hormones such as testosterone elevation in pubescent animals (Osório, Osório, & Sañudo, 2009; Lents et al., 2018). Therefore, the production of animals castrated and slaughtered early can be a tool to eliminate part of the factors that negatively influence the quality of the meat, promoting a greater acceptance of the product by the consumers.

As a way to overcome most of the disadvantages caused by surgical castration, immunocastration was created, a form of immunological sterilization using an anti-GnRH vaccine. The vaccine works as a suppressor of the gonadotropin-releasing hormone (GnRH), which plays a central role in regulating sexual function, resulting in the suppression of the secretion of gonadotropins LH and FSH in the anterior pituitary, and a consequent reduction in testosterone production in male gonads (Zanella et al., 2009).

Castration using an anti-GnRH vaccine has already been validated in studies with several animal species such as cattle, pigs, horses and wild animals (Janett et al., 2012). The hypothesis tested was that lambs submitted to immunocastration with anti-GnRH vaccine would present similar productive performance to that of non-emasculated animals, but with seminal and histological characteristics compatible with emasculated animals. Thus, the objective of this work was to evaluate the efficiency of the anti-GnRH vaccine in lambs regarding the productive performance, seminal characteristics and histological characteristics of the testicles.

Material and Methods

The experimental protocol was approved by the Ethics Committee on Animal Use at the Federal University of Mato Grosso do Sul (UFMS) (approval no. 862/2017).

The experiment was carried out at the School Farm of the Federal University of Mato Grosso do Sul, located in Terenos, Mato Grosso do Sul, from June to September 2018. Twenty contemporary Texel lambs, five months old, were used and average initial weight of 34.6 ± 6.17 kg. The experimental design was completely randomized and the treatments consisted of vaccinated and control animals.

The animals were distributed in the treatments using body weight as a criterion. They were allocated in modules formed by *Urochloa* spp., with fixed capacity, in which they remained in rotational grazing for 15 days in each paddock. All paddocks were provided with feeders for supplementation and drinking fountains with water at will. The supplement was formulated for consumption of 2% in relation to body weight and expected gains of 200 g/day, according to the requirements of the National Research Council [NRC] (2007). The immunocastrated and control animals were subdivided into two more groups, where 10 immunocastrated animals and 10 control animals, received supplement without extruded urea.

The animals were weighed at the beginning of the experiment and after 15 days of adaptation to the management experimental. After this period, weighing was carried out every 21 days to determine the weight gain and adjust the supplement supply.

The management for immunoscastration of the animals consisted

of the application of 1 ml of the anti-GnRH vaccine (BoPriva®) subcutaneously in the axillary region of the treated animals, at 30 and 60 days of experiment. On the same dates, the control animals received 1 mL of saline solution subcutaneously in the axillary region, so that they would go through the stress of the vaccine application.

Semen was collected from all animals by electroejaculation, on the day of the first and second doses of the vaccine and 30 days later (90 days of experiment). The evaluated characteristics were: sperm concentration ($\times 10^9$ mL), seminal volume (mL), swirling, motility (%) and sperm vigor. The collection procedures, physical and morphological evaluation of the semen were performed according to Brazilian College of Animal Reproduction [CBRA] (2013).

Blood collection management took place every 30 days, before the concentrate was delivered in the morning, through jugular venipuncture, and the samples were centrifuged to obtain serum for evaluation. Jugular vein puncture blood samples were collected with BD SST II Advance® vacuum tubes containing clot activator and separator gel and BD Vacutainer® Fluoride/EDTA tubes as a glycolytic inhibitor and anticoagulant EDTA. Tubes were centrifuged (3.000 rpm for 15 minutes) and the serum were stored in 2 mL polypropylene tubes, refrigerated and was evaluated total protein (kit ref. 04657586), urea (kit ref. 11200666), albumin (kit ref. 04657357), creatinine (kit ref. 10886874), alanine aminotransferase (ALT) (kit ref. 10745138) and aspartate aminotransferase (AST) (kit ref. 10745120).

The experiment was set up as a randomized-block design as a function on body weight, with ten repetitions per treatment, and analyzed by the F test, at a 0.05 level of significance, according to the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was the observed value for each variable, μ is the general mean; T_i is the effect of treatment ($j = 1, 2$) and e_{ij} is the random error associated with each observation.

Results and Discussion

At 30 days of experiment, in the application of the second vaccine dose and the second semen collection, there was a higher sperm concentration, ejaculate volume and sperm motility (Table 1), because, at that moment, the animals were six months old. age and, therefore, were close to puberty. At this time, even after 30 days of the application of the first dose of the anti-GnRH vaccine, it was not possible to verify changes in the seminal characteristics (Table 1), which was expected, since the animals would be considered effectively spayed after 30 days of application of the second dose of the vaccine. In the first semen collection, which occurred concomitantly with the application of the first dose of the vaccine, satisfactory values were not reached according to the values established by CBRA (1998) for the use of sheep breeders in the andrological characteristics evaluated due to the sexual condition of the animals, which at five months of age were not yet pubertal.

Table 1
Seminal characteristics of whole and immunocastrated lambs

	Treatment		SEM	P value*
	Imunocastrated	Control		
Sperm concentration (x10 ⁹ /mL)				
1st harvest	13.9b	23.0a	6.10	0.0037
2st harvest	24.1	25.4	6.90	0.6788
3st harvest	17.6b	26.2a	7.78	0.0238
Motility (%)				
1st harvest	12.5	18.5	21.38	0.5439
2st harvest	44.0	51.0	33.77	0.6486
3st harvest	4.5b	61.0a	17.50	0.0001
Force				
1st harvest	0.9	1.5	1.14	0.2546
2st harvest	2.3	2.6	1.67	0.6935
3st harvest	0.4b	3.0a	0.95	0.0001
Volum (mL)				
1st harvest	0.3	0.6	0.47	0.1850
2st harvest	0.6	0.9	0.48	0.1566
3st harvest	0.1b	0.7a	0.40	0.0023
Whirlwind				
1st harvest	0.0	0.0	-	-
2st harvest	0.0	0.0	-	-
3st harvest	0.2b	2.7a	0.93	0.0001

SEM = standard error mean;

* Means followed by lowercase letters, on the same line, differ from each other by the F test (P <0.05).

In the third semen collection, at 90 days, the control animals showed good quality semen, since, at that moment, they were seven months old and were closer to sexual maturity. For immunocastrated lambs, although sperm production still exists, semen was not viable for reproduction due to low motility (4.5%), vigor (0.4), swirling (0.2) and volume (0.1), according Table 1. Thus, at 30 days after application of the second dose of the vaccine, the animals showed a positive response to treatment, being unable to reproduce, that is, castrated.

According to Souza, Urt, Ítavo, Melo and Silva (2019), ejaculation of viable sperm, indicative of onset of puberty, occurs between four to six months of age in sheep, when the body weight of lambs corresponds to 40-60% of the adult animal. Corroborating the results of the study by Lents et al. (2018) with immunocastrated goats, which had azoospermia and swirling values, vigor, motility and concentration below those recommended by the CRBA, 60 days after the application of the second dose of the

anti-GnRH vaccine. Similar results have been reported in the studies by Janett et al. (2012) with cattle and those reported by Lugar et al. (2017) with goats, in which the vaccinated animals showed incomplete spermatogenesis or no sperm production after the application of the second dose of the vaccine.

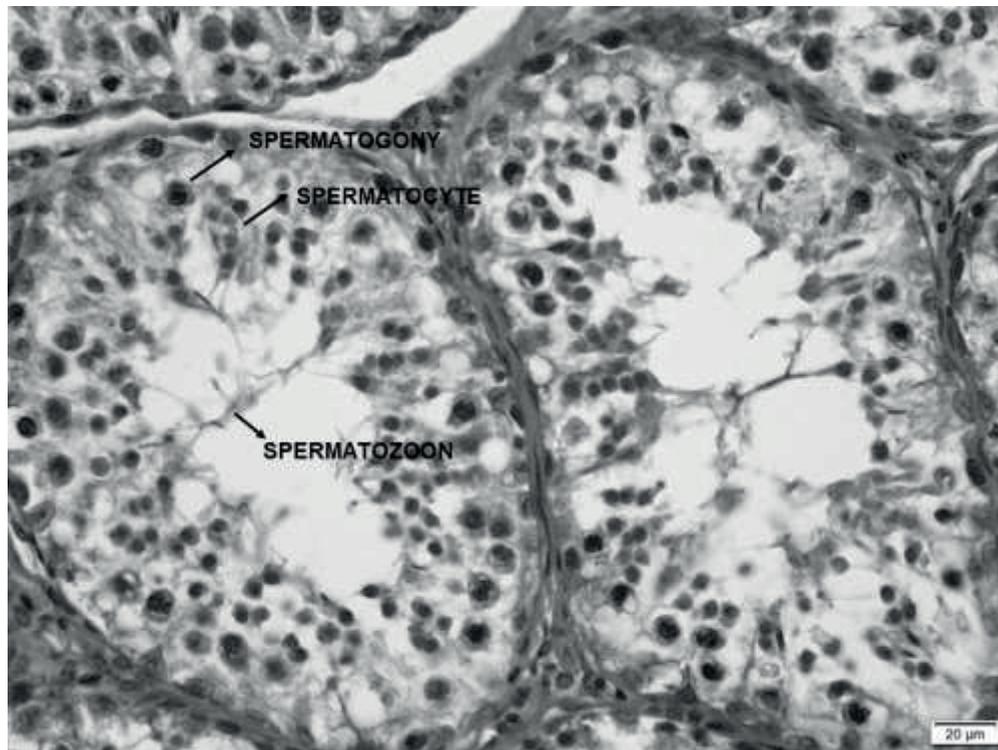
The weight of the testicles of the treated animals was significantly less than the weight of the control animals. The mean total weight was 90 g for immunocastrated lambs and 350 g for control animals. The right testicle had a mean of 50 g in immunocastrated lambs and 170 g in control animals, while the left testicle had a mean of 50 g for immunocastrated and 180 g for whole animals.

In an experiment with immunocastrated pigs, Andreo et al. (2018) affirmed that there is individual variation in the antibody titers generated by the vaccine among animals due to factors such as genetics, nutritional status, age and stress at the time of application, which explains the fact that in the present study animals have different responses to treatment. Gogić et al. (2019) with swine, which found a reduction of 28.9 to 54.4% in the testis volumes and weights of immunocastrated animals compared to control animals. According to Lents et al. (2018) these reductions in testicular volumes can be attributed to a reduction in the diameters of seminiferous tubules and the reduced mitotic capacity of sperm cells after the application of the anti-GnRH vaccine.

When comparing the aspect of the testicle of an entire animal, which showed complete spermatogenesis (spermatogonias, spermatocytes I, spermatocytes II, spermatozoa and spermatozoa) in the lumen of the seminiferous tubules, to the aspect of the testis of an animal that received doses of the anti-GnRH vaccine (Figure 1), it is noted that the animals that were immunocastrated developed fibrosis inside the seminiferous tubules and lost a large part of the spermatogenesis, causing a partial or total absence in the production of sperm (Table 1).

The histological characteristics observed in the study were confirmed by Zanella et al. (2009) who, when carrying out a study on immunocastration with the bovine species, also found testicular degeneration, desquamation of the seminiferous epithelium and absence of sperm production. Needham, Lambrechts and Hoffman (2019) and Han et al. (2015), both in experiments with sheep, claim that immunocastrated animals had a reduction in the diameters of seminiferous tubules and a reduction in the number of germ cells and each stage of development. In the study by Earl et al. (2006) with Suffolk sheep, observed that the immunocastrated animals showed thickening of the seminiferous tubule wall, enlargement of the basement membrane, small vessels, poorly defined interstitial cells and thickened connective tissue, while the control animals presented successive generations of sperm cells to from the basal lamina to the tubular lumen, meeting the results found in the present study.

A



B

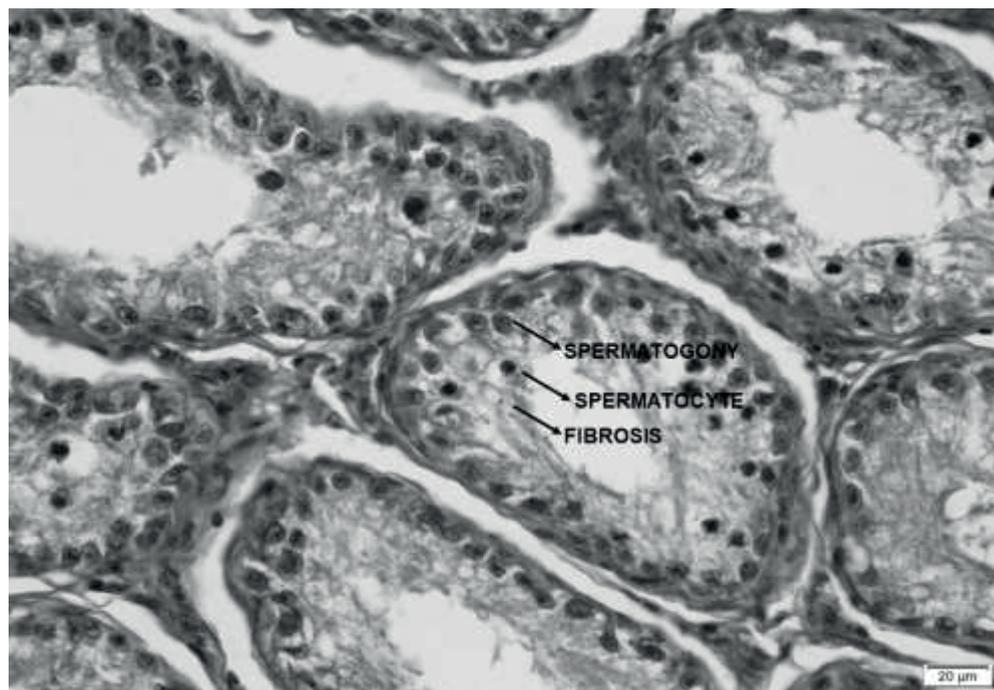


Figure 1. Histological characteristics of the testicles of the control (A) and immunocastrated lambs (B).

The control animals had a higher weight at slaughter, greater total and daily weight gain, but a worse feed conversion than the immunocastrated animals (Table 2), demonstrating that the performance of the immunocastrated sheep was inferior to that of the whole animals. The differences between whole and castrated animals have also been observed by other authors. Andreo et al. (2013), using immunocastrated and non-castrated cattle in confinement, observed a significant difference between treatments for final weight and daily weight gain. In contrast, Cook, Popp, Kastelic, Robbins and Harland (2000) working with immunocastrated and non-castrated cattle, did not observe any difference in the average daily gain of the animals during the rearing phase. However, in the finishing phase, the average daily weight gain was higher for non-castrated animals, compared to immunocastrated animals.

This difference may be related to the fact that, during the rearing, the animals were in the prepubertal stage or had not yet reached reproductive maturity, therefore, there was no significant interference of testosterone in the deposition of muscle and adipose tissue in the carcass. In the finishing phase, when the animals were at a more advanced chronological age and, consequently, more sexually mature, the effect of testosterone may have been pronounced, reflecting in the greater deposition of muscles in the carcass of whole animals in front of the castrated animals, the which resulted in higher average daily earnings. Whole animals have, in the composition of the gain, greater muscle deposition than castrated animals, the latter returning part of the energy ingested to the

deposition of adipose tissue, due to the absence of testosterone. The deposition of muscle tissue in the carcass is more energy efficient than the deposition of adipose tissue, justifying why castrated animals show less weight gain compared to whole animals, under the same age and conditions of rearing and management (Gómez et al., 2017).

Restle, Alves and Neumann (2000) observed in confined cattle, that the whole animals consumed 6.6% less food than the castrated ones to gain 1 kg in live weight, demonstrating the importance of testosterone produced by the testicles not only in weight gain, but also in feed efficiency.

Non-castrated animals have a higher weight at slaughter, greater total weight gain and greater average daily weight gain due to the higher blood concentration of testosterone, a steroid that provides an anabolic effect, promoting muscle hypertrophy of animals (Machado et al., 2018). Corroborating the studies by Janett et al. (2012) with cattle, in the present study the treated animals had a carcass similar to the control animals, however, control animals had a better feed conversion.

There was a difference for GGT values between treatments with immunocastrated animals between 55.9 IU/L and 58.9 IU/L, and for control animals, between 55.8 IU/L and 57.8 IU/L. There was a decrease in the AST value of the animals from the beginning to the end of the experiment, decreasing from 101.1 IU/L to 87.9 IU/L in the immunocastrated animals. The same behavior was observed in the whole animals, decreasing from 104.2 IU/L to 92.9 IU/L (Table 3).

Table 2
Performance of lambs finished in pasture as a result of immunocastration

	Treatment		SEM	P value*
	Imunocastrated	Control		
Slaughter weight (kg)	44.3b	48.3a	0.745	0.0001
Total weight gain (kg)	9.9	10.3	0.742	0.1200
Average daily gain (g/day)	104.5	108.9	0.781	0.1200
Average supplement consumption (g/day)	608.8b	642.7a	12.644	0.0001
Feed conversion	5.8	6.0	0.536	0.4325

SEM = standard error mean;

* Means followed by lowercase letters, on the same line, differ from each other by the F test (P <0.05).

Table 3
Biochemical profile of immunocastrated and control animals (group means)

	Treatment		SEM	P value*
	Imunocastrated	Control		
GGT 30 days	55.9	55.8	9.55	0.9628
GGT 60 days	58.9	57.8	10.95	0.6285
GGT 90 days	58.9	56.5	11.52	0.3527
AST 30 days	101.1	104.2	20.94	0.5059
AST 60 days	101.0	102.6	14.06	0.6011
AST 90 days	87.9	92.9	13.09	0.0937
Albumin 30 days	33.8	34.7	4.48	0.3719
Albumin 60 days	36.1b	38.0a	3.35	0.0132
Albumin 90 days	39.3b	41.5a	3.34	0.0056
Creatinine 30 days	0,9	0,9	0.14	0.2210
Creatinine 60 days	1.0	0,9	0.11	0.1395
Creatinine 90 days	1,0	1,0	0.09	0.0753
Total Proteins 30 days	64.0	64.6	4.45	0.5354
Total Proteins 60 days	70.1b	75.4a	3.06	0.0001
Total Proteins 90 days	74.2	75.3	4.55	0.2923
Urea 30 days	17.8	16.6	4.84	0.2955
Urea 60 days	28.7b	33.5a	6.44	0.0014
Urea 90 days	38.4b	45.5a	12.53	0.0135

SEM = standard error mean

* Means followed by lowercase letters, on the same line, differ from each other by the F test (P <0.05).

This reduction in levels of AST can be explained by Franciscato et al. (2006), who stated that in all domestic species the activity of AST is high in the liver, therefore, it does not always indicate an acute or chronic liver injury. Since in both treatments there was no significant difference between the values, it can be said that there were no losses in liver activity and in general physiology of control animals in relation to whole animals.

Conclusion

Lambs submitted to immunocastration with anti-GnRH vaccine had less productive performance than whole animals and had seminal and histological characteristics compatible with infertile animals.

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