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ORIGINAL ARTICLE

Effects of an anti-gonadoliberin releasing hormone vaccine on testicular, epididymal and spermatogenic development in the horse

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Abstract

The effects of the GnRH vaccine Improvac® on testicular and epididymal morphometrics, histology and spermatogenesis were measured in 19 young (15–20 months) colts randomly assigned to one control (saline, castration at 57 days, $n = 6$) or either of two GnRH vaccine-treatment groups, T-57 (castration at 57 days, $n = 7$) or T-100 (castration at 100 days, $n = 6$), respectively. All were immunized on Day 0 with a single booster on Day 28. Excised testes and epididymides were weighed and processed for histology to measure tubule, epithelial and muscle dimensions, the ratio of interstitial tissue to seminiferous tubules and determine the stage of spermatogenesis. Testis volume, unchanged within controls, decreased in T-57 and T-100 groups by 50% and 70%, respectively. Treated colts' testes were significantly lighter than controls (64% relative difference); however, epididymal mass showed no significant differences between groups. Proportionally less seminiferous tubule relative to interstitial tissue was observed in both treatment groups (5%) versus controls (22%) with a mean tubule size 28% smaller than controls. Controls exhibited a high proportion of seminiferous tubules with advanced stages of spermatogenesis, whereas treated colts showed a high proportion of tubules in the early stages of spermatogenesis. In conclusion, immunization against GnRH in prepubertal colts was effective at reducing the development of their intra-scrotal reproductive organs and preventing normal spermatogenesis. GnRH vaccination of young colts effectively and consistently reduced testis mass, tubule size and relative proportion of seminiferous tubule tissue while retarding spermatogenesis. The epididymis showed changes with a smaller tubule diameter, lower epithelial height and thicker muscle layer recorded in treated compared to control colts.

KEYWORDS
epididymis, GnRH vaccine, histological effects, testis

1 | INTRODUCTION

Reproductive function and associated behaviour has been controlled in stallions via surgical, hormonal, or immunological methods (Stout, 2001; Stout & Colenbrander, 2004). Historically, orchidectomy has been the most common method applied (Van der Velden, 2005), its major shortcoming being the loss of genetic potential prior to establishing a stallion's ultimate performance capacity (Janett et al., 2009). In addition, surgical castration, although effective in removing both spermatozoa and the major source of androgens, has various attendant surgical complications (Railton, 1999) and does not necessarily resolve all behavioural problems (Line et al., 1985).

Gonadotrophin releasing hormone (GnRH) plays a crucial role within this cascade, and, consequently, active immunization against GnRH has been documented since the early 1970s to result in immunocastration of male mammals (Arimura et al., 1973; Fraser & Gunn, 1973; Hodges & Hearn, 1977) including stallions and, in most cases, has proved to be a suitable alternative to surgical castration (Thompson, 2000). These include anti-GnRH antibody response, reduction in testicular dimensions and weight and effects on testicular histology. Deleterious effects on semen parameters have also been described (Dowsett et al., 1991, 1996; Janett et al., 2009; Malmgren et al., 2001; Turkstra et al., 2005).

An anti-GnRH vaccine (Improvac®, Zoetis) developed for immunocastration of male pigs (Dunshea et al., 2001) has been tested extensively in mares (Botha et al., 2008; Imboden et al., 2006; Schulman et al., 2012). It effectively induced anoestrus in mares and proved a suitable alternative to surgical ovariectomy for long-term control of cyclicity (Botha et al., 2008). The first study to use this vaccine in colts (Birrell et al., 2021) reported consistently high antibody titres, decreased testicular volume and undetectable serum testosterone concentrations by Day 28. We investigated the effects in these animals as treated with the anti-GnRH vaccine on testis and epididymal mass and histology at 57 or 100 days post-primary treatment.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study was approved by the Animal Ethics Committee of the University of Pretoria (V047/11) as a continuation of the study on the effects of an anti-GnRH vaccine on the post-surgical clinical outcomes at castration (Birrell et al., 2021). Briefly, 19 male light-horse types of various breeds, aged from 15 to 20 months, were randomly allocated to one of three study groups: a control group ($n = 6$, mean age 18 months) treated with 2 ml saline by intramuscular injection on Days 0 and 28 and surgically castrated on Day 57 post-primary treatment; T-57 group ($n = 7$, mean age 18.8 months) treated with 2 ml anti-GnRH vaccine containing 400 μg GnRH-protein conjugate on Days 0 and 28 and castrated on Day 57 post-primary treatment; and a T-100 group ($n = 6$, mean age 18.4 months) treated with 2 ml anti-GnRH vaccine on Days 0 and 28 and castrated on Day 100 post-primary treatment (Birrell et al., 2021). Inclusion criteria were general physical soundness, good body condition and normal scrotal contents on clinical examination.

2.2 | Testicular and epididymal mass

After excision, the epididymis was dissected from the testis; both were individually weighed (Radwag PS 6000/C/2, Radom, Poland) and processed immediately.

2.3 | Testicular and epididymal histology

2.3.1 | Tissue collection and processing

Testes were halved longitudinally with a transverse section of approximately 4 x 12 x 20 mm taken from the centre of each half. These sections were fixed in 4% buffered formalin (≥72 hr) and embedded in paraffin wax using standard protocols. After a number of sections had been cut from each testis block and discarded, a single 4 μm section was cut and stained with Mayer's haematoxylin and eosin.

From epididymal tissue, a transverse section was taken from the centre of each of both the left and right caput, corpus and cauda regions, respectively. The fixing, sectioning and staining procedures were similar to that for the testes.

2.3.2 | Testicular histological procedures

Sections were analyzed using an Olympus BX63 light microscope (Olympus Corporation) equipped with an Olympus cell Sense Imaging Software image analysis program (Olympus Corporation). Images were captured with an Olympus DP72 camera. Per colt, 50 locations in each testis (left and right; 100 per colt) were randomly selected using a motor-driven stage where the coordinates had been predetermined for each testis by an independent person. A minimum of 25 tubules were randomly selected in each testis (50/colt) to measure seminiferous tubule circumference (STC) and seminiferous tubule diameter (STD). Each section was given an individual number, which did not indicate either the identity of the colt or the treatment group of the colt. The operator was thus blinded to the identities of colts and their groups. For obliquely sectioned tubules the differences between the diameters of the major and minor axes of the ellipse were used to identify deviations from the perpendicular. Cross-sectional diameters in the x and y planes were measured as proxies for the major and minor axes, respectively. Tubules with differences of more than 10%, corresponding to a deviation of approximately 25°, were excluded from analyses. Four measurements of muscle thickness and epithelial height measurements were obtained

for each of these tubules. The proportion of tubule to interstitial tissue (tubule:interstitial ratio, TIR) along a standard transect was measured using lengths of the transect intersecting with seminiferous tubule tissue, interstitial tissue, or slide preparation artefact. The proportion of tubule to interstitial tissue (tubule:interstitial ratio, TIR) along a standard transect was measured using lengths of the transect intersecting with seminiferous tubule tissue, interstitial tissue, or slide preparation artefact.

The stage of spermatogenesis observed in each seminiferous tubule was scored according to Johnsen (1970), as modified for stallions by Turkstra et al. (2005). A 10-point system was applied according to germ cells being present or absent: score 1, no cells in tubular cross-section visible; score 2, Sertoli cells but no germ cells; score 3, only spermatogonia; score 4, only a few spermatocytes (<5) and no spermatids or spermatozoa present; score 5, no spermatozoa or spermatids, but several or many spermatocytes present; score 6, no spermatozoa and only a few spermatids (<5–10) present; score 7, no spermatozoa but spermatids present; score 8, few spermatozoa (<5–10) present; score 9, many spermatozoa present but germinal epithelium disorganized, with marked sloughing or obliteration of the lumen; and score 10, complete spermatogenesis with many spermatozoa, and an open lumen.

Additionally, the general subjective histological features of the sections were assessed. These observations included the presence, distribution, and appearance of Leydig cells, vacuolation in the tubule lumen and presence of lipofuscin granules.

2.3.3 | Epididymal histological procedures

Up to 10 suitable locations were selected in each section of the left and right caput, corpus and cauda epididymal regions to measure tubule diameter, muscle layer thickness and epithelial height. Measurements were similar to those described for the seminiferous tubules.

2.4 | Statistical analysis

Null hypotheses were a difference of zero between groups. Where the alternative hypothesis could include differences greater than or smaller than zero (as for changes in stage of spermatogenesis), two-tailed *t*-tests were used. In most cases, the literature reported a reduction in testis parameters following GnRH vaccination and therefore supported one-tailed *t*-tests being used. All quantitative analyses were conducted using R (R Core Team, 2016), and the BEST package (Kruschke & Meredith, 2015). For correlation, analysis between testis mass and STC or TIR, the posterior distribution for Pearson's product-moment correlation (P or *r*) was used. Differences were considered significant at $p \leq .05$.

For each animal, 50 tubules were measured from both the left and right testis. The mean of the vertical and horizontal diameters for each tubule was compared to the STC. The classical Pearson's product–moment correlation between diameter and circumference (both log-transformed) was 0.9967. Because of this exceptionally strong correlation, STC was used for further analyses of tubule size, but STDs are also reported for comparison with other studies. For each tubule, the medians of the two diameters and the four epithelium and four muscle thickness measurements, respectively, were calculated. For each testis, the median of the representative tubule measurements was calculated and subsequently the means of the left and right testis per colt.

The sections of the three regions of the epididymis yielded inconsistent numbers of suitable tubules for measurement, especially in the cauda region where no suitable tubules could be found in a number of sections. The means and *SEM* were thus calculated, and *t* tests were performed.

3 | RESULTS

The two treatment groups (T-57 and T-100) were compared to determine whether treatment duration had an effect on any of the morphological variables of the testes. No significant differences were found for testis and epididymal mass, STC, TIR and tubule score between the two groups, and data were thus pooled.

3.1 | Testis and epididymal mass

The mean testis mass of left and right testes of combined treated groups at castration was 28.62 g compared to 53.81 g for the control group, showing a significant 46.8% reduction (Table 1).

There was no statistical difference between the mean epididymal mass of the combined treated groups and the control group (Table 1).

TABLE 1 Mean testicular and epididymal masses of control and combined GnRH vaccine-treated colts on day of castration

	Group						
	Control			Treated			
Mass (g)	*n*	Mean (+*SEM*)	Range	*n*	Mean (+*SEM*)	Range	*p* value
Testis	6	53.73 (8.23)	35.9–89.9	13	28.52 (2.56)	16.5–39.9	<.001
Epididymis	6	25.92 (2.14)	15.5–43.5	13	33.07 (4.54)	21.2–49.0	.12

3.2 | Seminiferous tubule dimensions

The mean STC for the combined treatment groups showed a 28% relative reduction compared to the control group. Treated had smaller STDs than control colts, reflecting a 27% relative difference (Table 2).

3.3 | Relative proportions of testicular tissue components

The median proportions of seminiferous tubule and interstitial tissue are shown in Table 2. The combined treatment groups reflected a smaller TIR (0.89) than control colts (1.66), a relative difference of 47%. Expressed as a differential rather than a ratio, treated colts had a lower proportion of seminiferous tubule tissue relative to interstitial tissue than the control colts, which had 22% more tubules than interstitial tissue. Conversely, treated colts had 5% more interstitial than tubule tissue (Figures 1 and 2).

3.4 | Correlation between testis mass, seminiferous tubule dimensions and ratio of tubule to interstitial tissue

Correlations between testis mass and STC were significant, regardless of whether all colts combined were examined, or the treated or control groups separately. The correlations between testis mass and the TIR were smaller but nevertheless significant (Table 3).

3.5 | Spermatogenesis

In controls, a high portion of seminiferous tubules displayed a definitive lumen surrounded by a germinal epithelium containing all the cellular elements indicative of active spermatogenesis typical for normal testis parenchyma. A relatively thin layer of interstitial tissue invested the seminiferous tubules (Figure 1). In contrast, the testes of treated colts showed a reduction in seminiferous tubule size and morphological evidence of disrupted spermatogenesis, indicated by thinning of the germinal epithelium and disruption of the germ cell series. A marked increase in the thickness of the interstitial tissue was also observed (Figures 1 and 2).

Subjectively, clumps of Leydig cells were observed in the intertubule area of controls whereas in treated colts, Leydig cells were observed singly or in smaller groups (as aggregations of far fewer Leydig cells than seen in controls; Figure 1). Vacuoles were present in the lumenal region of seminiferous tubules in both the control and treatment groups but were particularly prominent in the treatment groups (Figure 1). Similarly, accumulations of lipofuscin granules were regularly observed in the interstitial tissue of both control (Figure 2) and treated colts.

Treated colts exhibited a higher proportion of seminiferous tubules in the early stages of spermatogenesis, whereas controls exhibited a greater number of tubules in the advanced stages of spermatogenesis (Figure 3). For the controls, tubules with many spermatids (score 7) were most prevalent, while only three of 13 treated colts exhibited tubules at this advanced stage of development (Figure 3).

In contrast, in treated colts, tubules containing only spermatogonia (score 3) were most prevalent and also in a higher proportion of those tubules. The proportion of tubules with many spermatocytes (score 5) and proportion of tubules with few spermatids (score 6) was low and did not differ between the control and treatment groups. A statistically significant difference was observed between treated and control colts in respect of the proportion of tubules with many spermatids (score 7) but not in tubules with only spermatogonia (score 3) or few spermatocytes (score 4) (0.03, 0.24 and 0.08, respectively).

3.6 | Epididymal tubule dimensions

The combined treatment groups displayed (with high certainty) a smaller tubule diameter, lower epithelium and thicker muscle layer than the control group, but only in the caput and corpus regions of the epididymis (Table 4; Figure 4). Although the mean tubule diameter and epithelial height were smaller and the muscle layer larger in

Morphometric measurement	Group		*p* value
	Control (±*SEM*)	Treated (±*SEM*)	
Seminiferous tubule dimensions (μm)			
Seminiferous tubule circumference	383 (40.41)	274 (16.74)	<.01
Seminiferous tubule diameter	116 (12.04)	85 (5.08)	<.01
Proportion of seminiferous tubule and interstitial tissue (%)			
Seminiferous tubule tissue (%)	56 (1.65)	43 (2.14)	
Interstitial tissue (%)	34 (2.69)	50 (2.56)	
Tubule:interstitial ratio	1.66 (0.17)	0.89 (0.11)	.001

Note: Measurements were carried out on 50 tubules per colt.

TABLE 2 Mean seminiferous tubule dimensions and median proportion of seminiferous tubule and interstitial tissue, and mean seminiferous tubule and interstitial tissue as a percentage of control ($n = 6$) and combined GnRH vaccine-treated colts ($n = 13$) at castration

the treated than in the control group, the sample numbers were too small for a meaningful statistical analysis. Where cauda tubules were visible in the sections of three control colts, sperm were present in each animal (Figure 4; Cauda; C). No sperm were identified in the cauda tubules of four T-57 and three T-100 colts (Figure 4; Cauda; T-57 and T-100).

4 | DISCUSSION

We investigated the effects of anti-GnRH vaccine on testis morphometrics in colts, particularly at the histological level. Previously, several GnRH vaccines were reported in stallions with a variable degree of success. Several factors complicated any comparison of these results and included small sample sizes, variable antibody response, observation duration, end points and methodologies used to assess testicular size and histology. In this study, treated colts developed rapid, marked antibody responses to anti-GnRH vaccine treatment (Birrell et al., 2021). In addition, compared to T-57 colts, the T-100 group showed a marked decline in titres from Days 23 to 57, a difference that did not affect the outcome on testis mass. Serum testosterone measurements in treated colts reached baseline concentrations by Day 24 post-primary vaccination, remaining there until castration on either Days 57 or 100 (Birrell et al., 2021).

Suppression of gonadotrophic hormone secretion following anti-GnRH vaccination was clearly reflected by the decrease in seminiferous tubule size and TIR. The difference in STD between the treated and control groups in the current study was comparable to the findings of Turkstra et al., (2005), but greater than that reported by Malmgren et al., (2001). GnRH vaccination reduced STC, a parameter not previously reported.

The current study observed a greater reduction in the relative proportion of tubule to interstitial testicular tissue in treated versus control colts than previously observed, irrespective of either vaccine administration route (Dowsett et al., 1991) or dose (Dowsett et al., 1996).

The effect of anti-GnRH vaccination on testicular function was clearly evident microscopically. Retarded spermatogenesis was displayed by the prevalence of lower Johnsen scores in the treatment group. Turkstra et al., (2005) reported much higher Johnsen scores and the presence of some spermatozoa following treatment. Given that approximately 7 weeks is required to complete one spermatogenic cycle (Swierstra et al., 1974), the 8-week interval from primary immunisation to castration of T-57 colts was sufficient for the vaccine to achieve its full impact. Delaying castration by a further 7 weeks in T-100 colts showed no additional effect on the Johnsen score. However, this would have similarly applied in the Turkstra

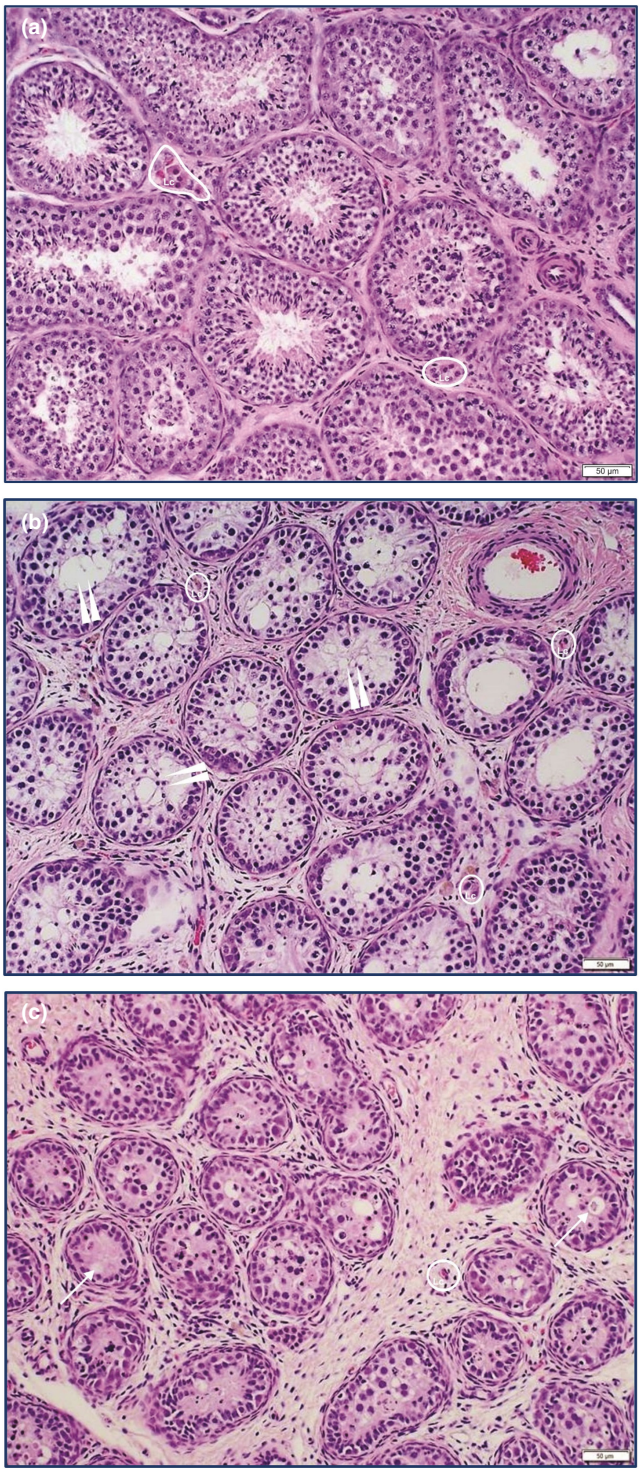

FIGURE 1 Light micrograph of the testis of one control ((a); castration Day 57) and two treatment colts castrated at 57 (b) and 100 (c) days, respectively; bars = 50 μm. (a): a number of seminiferous tubules are seen in the transverse section and display an active germinal epithelium consisting of, from the basement membrane inwards, spermatogonia, primary spermatocytes, secondary spermatocytes/round spermatids, and elongated spermatids. No spermatozoa are present in the tubule lumen. Note the relatively small amount of interstitial tissue surrounding the tubules. Clumps of Leydig cells (encircled) are present within the intertubule area. (b and c): Note the retarded spermatogenesis, the relative reduction in tubule diameter, and the increased amount of interstitial tissue compared to (a). Randomly located vacuoles, either singly (arrows) or in groups (double arrowheads), are present within the seminiferous epithelium. Single Leydig cells (encircled) are present within the interstitial tissue

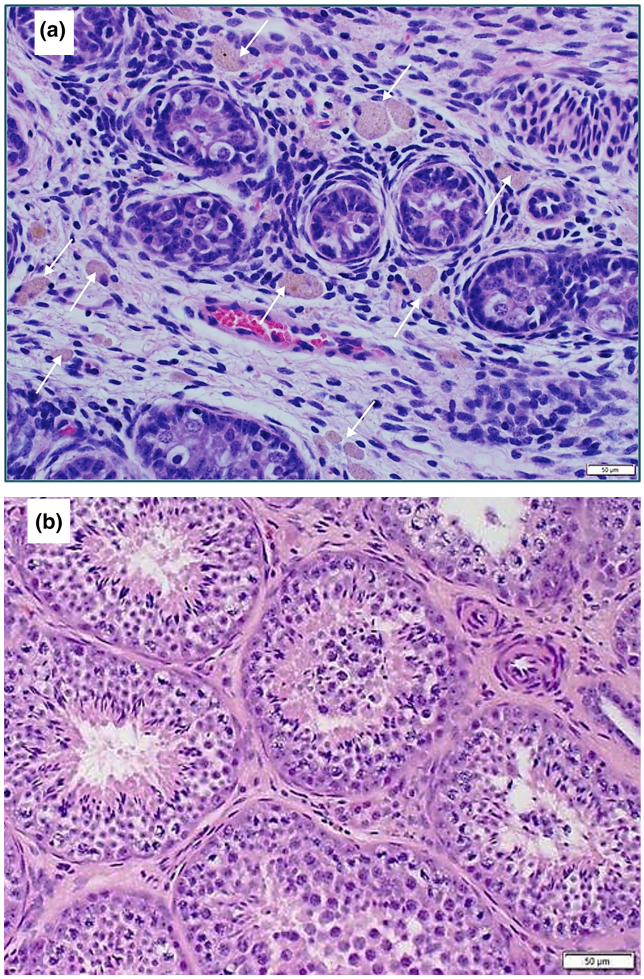

FIGURE 2 (a): Light micrograph of a cross-section of the testis of an anti-GnRH vaccine-treated colt. Large accumulations of lipofuscin granules (arrows) are observed in the interstitium. Accumulations of lipofucsin granules are normal occurrences in developing testicular tissue. (b): Light micrograph of a cross-section of the testis of a control colt with no visible lipofuscin granules (Bars = 50 µm)

et al. (2005) study with castration after 96 days. Spermatogenic activity affects individual ST size, clearly shown in this study. Thus, unsurprisingly, testis mass was well correlated with both STD and TIR, although the correlation of the latter was weaker.

Similar to previous observations (Dowsett et al., 1991, 1996), histologically, in controls the clumps of Leydig cells in the intertubule tissue were observed, whereas in treatment colts, they were seen singly or in small groups. Turkstra et al., (2005) reported the presence of atrophied Leydig cells in all and Malmgren et al. (2001) in two of three vaccinated stallions, respectively. These effects could be ascribed to a lack of LH stimulation. In addition, decreased testosterone concentrations in the treated colts (Birrell et al., 2021) reflected the smaller number of Leydig cells observed. The significance of luminal vacuolation in the seminiferous tubules in both control and treated colts (Figures 1b,c and 3b) was most likely related to the development of the lumen, which takes place during puberty (Pearl et al., 2011; Rode et al., 2015). The presence of accumulations of lipofuscin granules observed in all colts (Figure 2) was also described by Malmgren et al., (2001) and is reportedly normal for developing tissue of pre- (3–11 months) and peri-pubertal (12–23 months) stallions (Pearl et al., 2011).

TABLE 3 Pearson's correlations between testis mass (log-transformed) and either tubule circumference (log-transformed) or tubule to interstitial tissue ratio (log-transformed) for all colts, controls ($n = 6$), and GnRH vaccine-treated (T-57 plus T-100; $n = 13$) colts

Comparison	LCI	*p*	UCI	r^2	*p value*
Tubule circumference					
All	0.73	.89	0.96	0.70	<.001
Control	0.26	.88	0.99	0.78	.02
Treated	0.44	.80	0.94	0.64	.001
Tubule to interstitial tissue ratio					
All	0.41	.73	0.89	0.53	<.001
Control	−0.17	.75	0.97	0.56	.09
Treated	−0.09	.48	0.82	0.23	.09

Note: The correlation is indicated by *p*, bounded by upper (U) and (L) lower confidence intervals (CI; 95% CI). Fit is indicated by r^2. Number of tubules measured per colt = 50.

The suppression of spermatogenesis reflected in the current study was greater and notably more consistent than in previous studies. While this may have been related to the age of the colts, there are indications that the gonadal effects of the GnRH vaccine used appeared greater than that of previously used antigens or vaccine formulations (Dowsett et al., 1991, 1996; Malmgren et al., 2001; Turkstra et al., 2005). The anti-GnRH vaccine used in this study, was first tested in elephant bulls in 2006 for the suppression of androgen-related aggressive behaviour and musth (De Nys et al., 2010). The results were encouraging and now the vaccine is routinely used to control aggression, especially in captive bulls (Bertchinger & Sills, 2013; Rajapaska et al., 2010; Somgrid et al., 2015). In addition, GnRH vaccination in elephants markedly reduces semen quality and the size of the internal reproductive organs (Bertschinger & Lueders, 2018; Lueders et al., 2017). In this regard, this treatment's efficacy demonstrates the potency of the vaccine.

The effects of GnRH vaccines on epididymal weight and histology have not, to the authors' knowledge, been previously described in any mammalian species. In this study the decrease in mean post-castration epididymal mass of the treated colts was insignificant (0.12). The decrease was accompanied by a significant decrease in caput and corpus tubule diameter. Epithelial height also decreased and muscle layer thickness increased relative to the controls. The observed changes were ascribed to the probable decrease in testosterone metabolites essential for normal epididymal function and which reach the tubules via the lumen and blood supply. The sperm present in cauda tubule sections of controls were, however, absent in the treated colts.

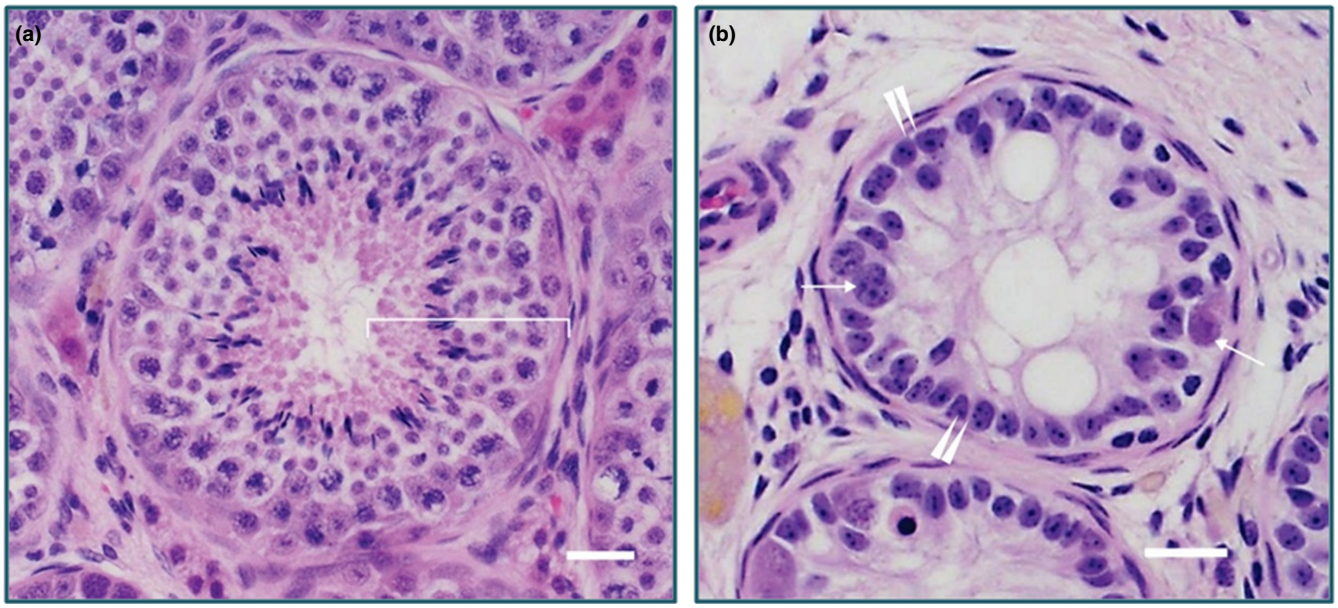

FIGURE 3 Light micrographs of seminiferous tubules of a control (a) and anti-GnRH-treated colt (b) at castration on Day 57 (T-57). (a): The seminiferous epithelium (bracket) displays all the cell stages typical of normal spermatogenesis. (b): Retarded spermatogenesis is evident and spermatogonia (single arrows) are the only germ cells present. The vertically oriented nuclei (double arrowheads) indicate Sertoli cells. Note the large number of vacuoles occupying a luminal position (Bar = 20 μm)

TABLE 4 Comparison of the mean tubule diameter, epithelial height, and muscle thickness of the caput, corpus, and cauda regions of the epididymides of sontrol and combined treatment (T-57 plus T-100) colts castrated on days 57 (control and T-57) or 100 (T-100)

		Control ($n = 6$)			T–57 plus T–100 ($n = 13$)					
Variable	Region	*n*	mean	*SEM*	*n*	mean	*SEM*	Difference	Change	*p*
Diameter	Head	6	352.19	13.106	11	283.87	10.06	68.32	Smaller	.01
	Body	6	330.48	11.839	13	260.46	6.23	70.02	Smaller	<.001
	Tail	3	437.08	30.15	7	389.49	35.82	47.59	Smaller	.394
Epithelium	Head	6	53.05	6.451	11	31.84	1.88	21.21	Smaller	.25
	Body	6	52.2	3.258	13	39.35	1.92	12.85	smaller	.11
	Tail	3	44.98	2.769	7	30.54	1.18	14.44	smaller	.73
Muscle	Head	6	42.48	3.474	11	56.38	1.47	−13.9	larger	.1
	Body	6	36.04	1.418	13	46.64	1.26	−10.6	larger	.02
	Tail	3	65.95	7.07	7	73.38	5.70	−7.43	larger	1.0

Note: n, number of colts contributing to each variable.

5 | CONCLUSIONS

This was a pilot study and was limited by the experimental population consisting of a relatively small sample size. Blocked randomization by age or weight could have been performed in order to create more equal groups at the start. The effects of anti-GnRH vaccination on colts were more marked and consistent than in previously reported GnRH-vaccine studies. Anti-GnRH vaccination in young colts effectively reduced testis volume, mass, seminiferous tubule size and relative proportion of seminiferous tubule tissue while additionally retarding spermatogenesis. It should be noted, however, that compared to most other studies, the colts used in the present study were much younger and the groups more homogeneous. The lower anti-GnRH antibody titres observed in Group T-100 compared to T-57 (Birrell et al., 2021) did not influence testis mass at castration. These effects in older stallions may not be as marked nor as consistent. Further research is indicated to investigate the long-term effects of anti-GnRH vaccination in mature stallions.

AUTHOR CONTRIBUTIONS

Alma E. Botha contributed to conceptualization, methodology, investigation including histology, data curation, writing—original draft (PhD chapter) and editing, and project administration. Martin L. Schulman contributed to conceptualization, methodology, investigation, writing and editing—co-supervision. John Birrel contributed

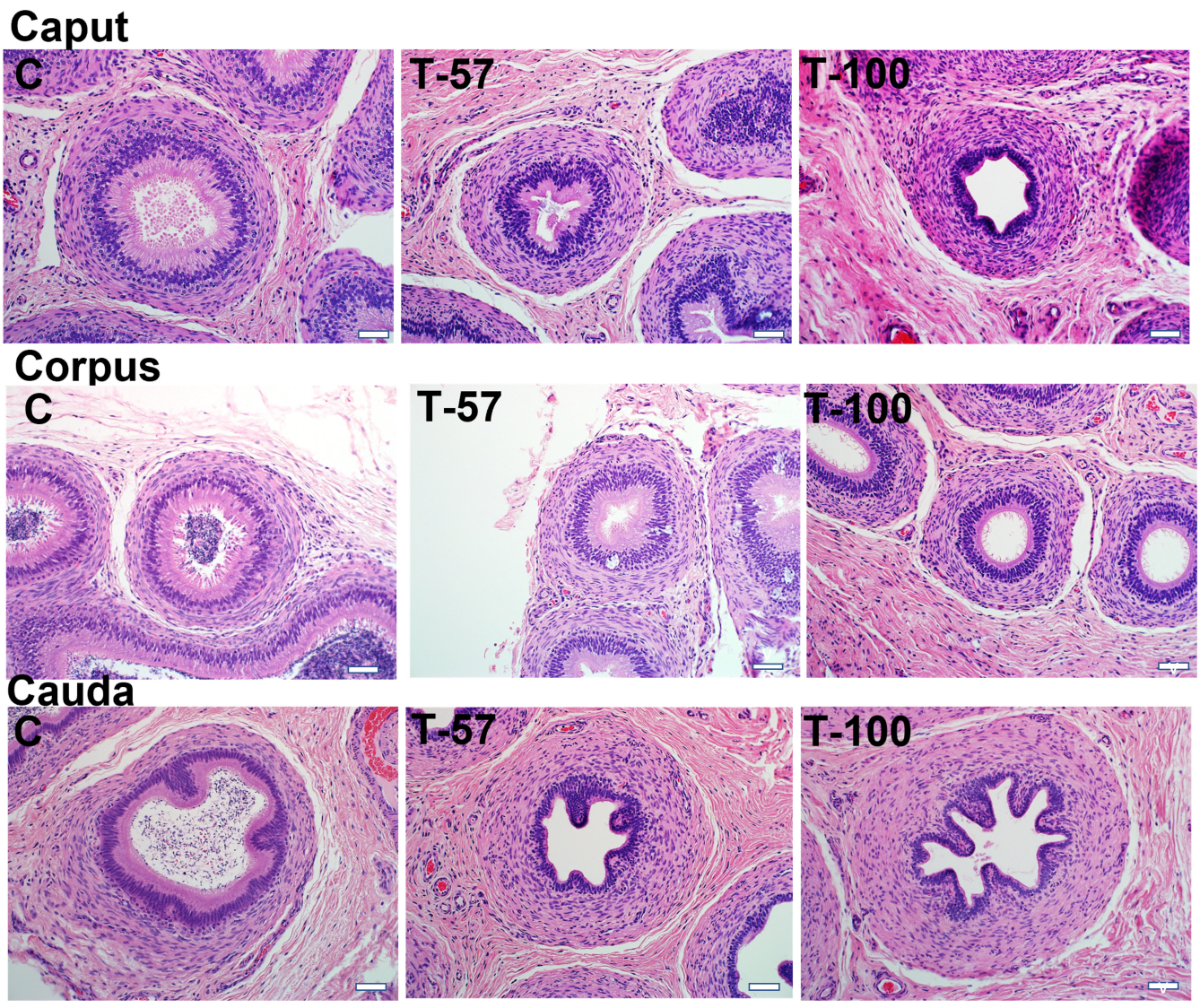

FIGURE 4 Caput: caput tubules of control (C) and treated colts castrated on Day 57 (T-57) or on Day 100 (T-100), respectively. Bar = 50 μm. Corpus: corpus tubules of control (C) and treated colts castrated on Day 57 (T-57) or on Day 100 (T-100), respectively. Bar = 50 μm. Cauda: cauda tubules of control (C) and treated colts castrated on Day 57 (T-57) or on Day 100 (T-100), respectively. Note the sperm present in the tubule of the control colt (C). Bar = 50 μm

to resources—colt acquisition, management and provision of materials, methodology, and investigation. Lizette du Plessis contributed to histological sections, histology and microphotography. Peter N. Laver contriubted to statistical analyses. John Soley contributed to methodology, histological interpretations and editing. Ben Colenbrander contributed to editing and histological interpretations. Henk J. Bertschinger contribute to conceptualization, methodology editing and supervision.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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