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Relationship between testosterone and penile spicules in Guinea pigs (*Cavia porcellus*)

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ABSTRACT

Understanding the physiology of penile spicules in guinea pigs (*Cavia porcellus*) may improve their management in large-scale production guinea systems. Here we examined whether penis spicule development depends on testosterone and whether exogenous testosterone can reverse penile spicule atrophy in castrated guinea pigs. The relationship between total testosterone (TT) and the development of penile spicules (DPS) in guinea pigs was explored in two experiments. The first experiment described the TT and DPS curves in non-castrated guinea pigs **(E1-G1)** and guinea pigs castrated on day 35 **(E1-G2)**. In the second experiment, the dose-dependent effect of the administration of exogenous testosterone **(ET)** was evaluated during DPS in **Group 1 (E2-G1;** castrated guinea pigs + 125 μg ET on days 65 and 80 of age), **Group 2 (E2-G2;** castrated guinea pigs + 250 μg ET on days 65 and 80 of age), **Group 3 (E2-G3;** non-castrated guinea pigs), and **Group 4 (E2-G4;** castrated guinea pigs without ET). Analysis of variance using a General Linear Model (GLM) was performed. TT increased from day 20 to day 35 in both groups in the first experiment ($P > 0.05$). This increase in TT was maintained in **E1-G1** on days 50, 65, and 80; however, TT fell to basal values in **E1-G2** after castration. DPS guinea was directly related to TT level. In Experiment 2, guinea **E2-G1** and **E2-G2** animals that received ET showed an increase in TT, significantly differing from **E2-G4** ($P < 0.05$). Nevertheless, ET administration in **E2-G1** and **E2-G2** was not sufficient to reach the TT levels in **E2-G3**. DPS was closely related to TT levels, such that when testicles were removed, the spicules began to atrophy without disappearing. Our results suggest that TT in guinea pigs increases steadily until puberty completes, after which it decreases and stabilizes and shows an association with DPS. Furthermore, 12–35% of TT produced by guinea pigs is testicle-independent. Finally, ET administration can stop and reverse the spicule atrophy process in castrated males. These results will help to manage guinea pigs in a more sustainable way in countries where this species is of utmost relevance to provide the population with the meat of high quality.

1. Introduction

Testosterone, a steroid hormone produced by testicles and adrenal glands, is present in male and female mammals, where it performs essential functions during pre- and postnatal development of the reproductive and urogenital systems. It exerts multiple influences at all stages of development after sex determination. In the males of most mammalian species, the *sex-determining region Y chromosome* (*Sry*) gene determines testis development [1]. This gene is thought to influence the transcription of genes involved in the differentiation of Sertoli cells. Subsequently, male differentiation requires the secretion of three testicular hormones: anti-Mullerian hormone, produced by fetal Sertoli cells; insulin-like 3, which mediates transabdominal testicular descent into the scrotum; and testosterone, produced by Leydig cells, which promotes the development of Wolffian duct derivatives and masculinization of the external male genitalia into the epididymis, deferent duct, and seminal vesicle during the embryonic stage [2]. Subsequently, the influence of testosterone is evident in the

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development of reproductive organs before puberty and gamete production after puberty [3,4]. Furthermore, its levels show nonlinear relationships with emotion, cognition, and copulatory reproductive behavior in animals [5].

One study reported that testosterone in the guinea pig presents a significant increase from day 16, when the prepubertal stage begins, up to day 60, when puberty is complete. Subsequently, testosterone decreases by 25% between days 60–90 and finally stabilizes [6]. This increase in testosterone is considered to be related to the development of the testes and other accessory organs such as seminal vesicles [7].

Many rodents have spicules or keratinized spines on their penis as part of their reproductive anatomy. These spines develop most likely from puberty, under the influence of sexual hormones, and constitute part of their reproductive strategy, although their specific function is unknown. In rats (*Rattus*), testosterone levels show an apparent relationship with growth of spicules, penis, and testicular tissue. In general, spicules are absent at birth, and they begin to grow towards the periphery of the penile epithelium at 42 days after birth, coinciding with the first rise in testosterone [8].

In sexually experienced male hamsters (*Mesocricetus auratus*), gonadectomy induces regression of penile spicules. Conversely, post-castration administration of androstenedione or, to a lesser extent, testosterone stimulates partial growth of penile spicules [9]. The Degú (*Octodon degus*) has thorns and spicules; the latter appear earlier after birth and shrink with age. This evolution is considered testosterone-dependent, and it is an indicator of androgenic and reproductive function [10].

In guinea pigs (*Cavia porcellus*), spicule removal reduces testosterone levels in the blood and inhibits development of the seminal vesicles [7]. This would imply that the spicules and testosterone act retroactively, in guinea pigs. Despite the importance of spicule development and physiology for guinea pig conservation and exploitation for bio- and zootechnology, relatively little is known about these structures or their potential relationship with testosterone. Understanding spicules better may improve the sustainability of guinea pig management, since in other rodents they mechanically trigger ovulation and therefore are essential for fertilization. Moreover, removing spicules from the guinea pig's penis may be a less invasive alternative to castration, facilitating reproductive management in animal production systems. The sustainability of guinea pig production systems is of great interest in South America and other less affluent regions, where the animals are prized for their high meat quality [11]. In addition, the animals are effective preclinical models in biomedical research [12].

In the present study, we explored the relationship of exogenous and physiological testosterone with the development of penile spicules in guinea pigs. We hypothesized that, as in other rodents such as rats, testosterone influences the development of spicules on the guinea pig penis. We further speculated that injection of exogenous testosterone might reverse penile spicule atrophy in castrated guinea pigs.

2. Materials and methods

This study included two experiments involving guinea pigs from an experimental farm at the University of Cuenca, Ecuador.

2.1. Animals and farm

All the research was carried out at the experimental farm of the University of Cuenca, Ecuador (altitude, 3100 masl; temperature, 12–18 °C; relative humidity, 75%). A group of 130 guinea pigs (Cavia porcellus) of the Perú genotype [13] were randomly selected for the study. Their age at the beginning of the experiments was 15–20 days, and their average weight was 204.4 ± 0.15 g. The animals were kept in mesh cages (1 × 0.50 m) and they received 12 h of light and 12 h of darkness per day. They were fed a diet of 60% ray grass (*Lolium perenne*), 20% kikuyo (*Pennisetum clandestinum*) and 20% commercial food. Guinea pigs were managed according to the ethical principles and regulations in Chapter 7.8 ("*Use of animals in research and education*") of the Terrestrial Animal Health Code of the World Organization for Animal Health [14].

2.2. Experimental design

2.2.1. Experiment 1

This experiment aimed to describe the testosterone curve, the development of penile spicules and the relationship between the two, throughout neonatal development, prepuberty, and puberty. Fifty guinea pigs were randomly distributed into two experimental groups:

- **Group G1 (E1-G1;** n = 25): non-castrated guinea pigs or control group.
- **Group G2 (E1-G2;** n = 25): guinea pigs castrated at day 35 of age.

Castrated animals were surgically castrated on day 35 of age as described [15].

For sampling, five individuals per experimental group and sampling day were randomly sampled and euthanized (Table 1). Thus, group size decreased after each sampling.

2.2.2. Experiment 2

In this part of the study, our objective was to evaluate the dose-dependent effect of the administration of exogenous testosterone on the development of penile spicules in previously castrated guinea pigs at late puberty. A total of 80 guinea pigs at 35 days of age were randomly selected and assigned to four experimental groups:

- **Group 1 (E2-G1):** castrated guinea pigs + administration of 125 μg of Testosterone Enanthate (Primoteston Depot®; Bayer, Veracruz, México) i.m., on days 65 and 80 of age; n = 20. Timing of testosterone administration was decided based on the results of Experiment 1, where maximal testosterone secretion was observed on day 65, when puberty completed. Our goal was to ensure a sufficient period when the castrated animals were not exposed to testosterone. This design also allowed us to detect whether non-testicular production of testosterone increased on day 65, as observed in Experiment 1.
- **Group 2 (E2-G2):** castrated guinea pigs + administration of 250 μg of Testosterone Enanthate (Primoteston Depot®) i.m., on days 65 and 80 of age; n = 20.
- **Group 3 (E2-G3):** non-castrated guinea pigs (control 1); n = 20.
- **Group 4 (E2-G4):** castrated guinea pigs without the administration of exogenous testosterone (control 2); n = 20.

Testosterone doses were decided based on previous studies [16,17] in which doses of 100–800 μg of testosterone were administered subcutaneously to castrated male Wistar rats in order to observe effects on the reproductive system and accessory glands. The repetition of administration at an interval of 15 days in the present study was based on the recommendations of the testosterone manufacturer.

The sampling for the assessment of DPS and TT in Experiment 2 was carried out on five randomly chosen guinea pigs per group and sampling day (days 35, 50, 65, and 90 of the experiment), in a similar way as previously described for Experiment 1.

Table 1
Protocol for the assessment of total testosterone (TT) and the development of penile spicules (DPS) in guinea pigs over time guinea (Experiment 1).

	Day of sampling				
	Day 1	Day 2	Day 3	Day 4	Day 5
Age of the animals	20d of age	35d of age	50d of age	65d of age	80d of age
Animals Sampled per group	5	5	5	5	5
Remaining animals per group	20	15	10	5	0
Evaluation	DPS + TT	DPS + TT	DPS + TT	DPS + TT	DPS + TT
Castration	-	G1-E2	–	–	–

DPS = Measure of the development of spicules of the penis. TT = Assessment of the total testosterone level. G1-E2 = castrated group. G1-E1 = non-castrated groups. Monitoring time points occurred at 20, 35, 50, 65 and 80 days of age.

On day 35 of the experiment, after a clinical review of testicular presence, a first assessment of the DPS and TT was made on five guinea pigs per group. These values served as a baseline to show the behavior of DPS and TT after administration of the treatments in the different groups; at the end of the first evaluation (day 35), these guinea pigs (n = 5) were euthanized. Immediately afterward, 15 guinea pigs from each of the E2-G1, E2-G2, and E2-G4 groups were castrated. The remaining animals from the group E2-G3 (n = 15) served as non-castrated control animals.

2.3. Castration, sampling and laboratory methodology

2.3.1. Castration methodology

Castration was performed according to the abdominal technique as described [15]. In brief, a skin incision (3–4 cm) was made in the ventral midline at the cranial pole of the bladder. The subcutaneous tissue was directly dissected. An incision was made in the alba line to access the abdominal cavity. Then, the testicle was visualized, and the tail of the epididymis of the hemy scrotal sac was dissected. The testicle was exteriorized, and the proximal part of the testicular cord was doubly clamped and sutured using 3–0 polydioxanone. The spermatic cord and testicle were removed. After confirming adequate hemostasis, the pedicle was released into a physiological position. The same procedure was repeated on a contralateral testicle. The muscular layer was continuously sutured with 3–0 polydioxanone, and the skin was closed using a continuous intradermal suture with 4–0 poliglecaprone (Monocryl; Ethicon). Adhesive (Vetbond tissue adhesive; 3 M Health Care) was added as needed, and wounds were sprayed with a permeable spray.

2.3.2. Blood sampling

Guinea pigs were anesthetized as described [18] using subcutaneous injection of a mixture of zolazepam and tiletamine (Zoletil 50®) at a dose of 50 mg/kg. With a 21-gauge, 25-mm vacutainer needle at a 30° angle, cardiac puncture was performed in the space between the third and fourth rib proximal to the sternum on the left side of the animal. Blood (1 mL) was obtained and allowed to clot. Samples were centrifuged at 2500 *g* for 15 min. The supernatant (serum) was taken and frozen at −20 °C until analysis.

2.4. Assessment of DPS development

This assessment was carried out on anesthetized, depilated guinea pigs. The penis was exposed and digital pressure was applied to visualize the spicules (Fig. 1). The spicules were then fixed and dissected with a clamp, placed in 2.5% formalin solution, and transported to the laboratory. Each spicule was deposited in a 35 × 15 mm Petri dish and analyzed under a stereoscope (SMZ 745, 123 Nikon, Tokyo, Japan) equipped with a high definition camera (Excelis AU-600-HD). Measurements (in μm) were obtained after processing the images with software (AmScope, version 3.7).

2.5. Euthanasia methodology

Once the samples were taken and the spicules were removed, each animal (five animals/group and sampling day in Experiments 1 and 2) was humanely euthanized as described [15]. In brief, guinea pigs were sedated with 40 mg/kg of ketamine (ketamine HCL; Hospira) and 2 mg/kg of xylazine (Rompun 2%; Bayer, Mississauga, Ontario, Canada), then immediately injected intracardiacally with Embutramida T-61 euthanasia solution (0.3 ml/kg body weight; Hoechst-Roussel Agri-Vet, Somerville, NJ, USA).

2.6. Total testosterone (TT) assessment

TT was determined with a commercial enzyme-linked immunosorbent assay test (Testosterone Kit ELISA, Neogen Corporation, ISO 9001, USA). Results were expressed in ng/mL. Analytical sensitivity was <0.006 ng/ml and the inter- and intra-assay coefficients of variation were ≤10%.

2.7. Statistical procedures

Data were analyzed using SAS (version 9.4). Data on testosterone concentration and length of spicules did not fulfill normality assumptions based on the Shapiro-Wilk test, so they were $\log_{10}$-transformed to allow analysis of variance using a General Linear Model (GLM). In both experiments, the effects of treatments, period, and repetition were considered. Differences between means were analyzed using the least-squares method, and values of $P < 0.05$ were considered to indicate significant statistical differences. Correlation between the development of penile spicules and testosterone was established using the Spearman test.

3. Results and discussion

The results obtained are described separately for each experiment.

3.1. Experiment 1

3.1.1. Total testosterone (TT)

Total testosterone increased gradually from day 20 to day 35 of age, in similar proportions in the two experimental groups ($P > 0.05$; Table 2). These results coincide with previous work [6], where the prepubertal period was defined from 16 to 60 days after birth, during which plasma testosterone increased linearly over time.

The sustained increase in TT was maintained in the E1-G1 group on days 50, 60 and 80; however, guinea pigs in the E1-G2 group (castrated at the age of 35 d) showed a decrease in TT to basal values (0.49 ± 0.33 ng/mL) on day 50 after castration. Similar to our results, a previous study [7] found that initial TT levels in chemically castrated guinea pigs (0.703 ± 0.03 ng/mL) fell to 0.55 ng/mL

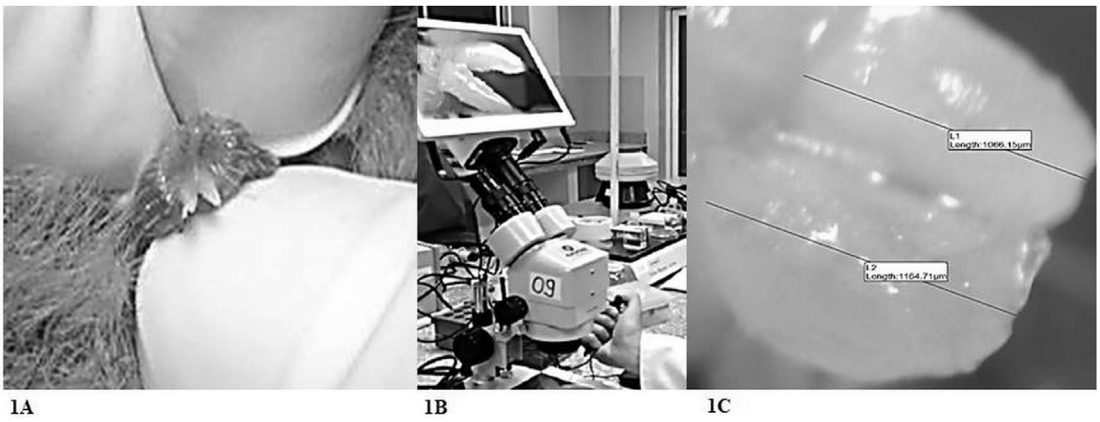

Fig. 1. Protrusion of guinea pig penile spicules (1A). Stereoscope image of penile spicules (1B). Process for measuring the development of penile spicules (1C).

at 7 days after administration of the chemical. Testosterone did not disappear completely in the present study (0.49 ± 0.33 ng/mL) or a previous study (0.55 ng/mL), possibly reflecting hormone production by the adrenal glands [4]. More than 95% of testosterone in men is produced by the testicles, while a zone of the adrenal cortex in both sexes produces small amounts of androgens [4]. Another study [19] described that the reticular fasciculate area of the adrenal glands in various animal species secretes glucocorticoids and small amounts of gonadal steroids as estrogens and androgens. In our guinea pigs, the relative level of non-gonadal testosterone release was 20–30% of the serum testosterone.

At the evaluation on day 65 (a prepubertal stage in guinea pigs), animals of the E1-G1 group exhibited the highest levels of TT, which differed significantly from the levels in E1-G2 ($P < 0.05$). However, even in the E1-G2 group (castrated animals), an increase was observed in TT levels upon completing puberty (day 65) (Table 2). These data agree with previous work [20] in which the highest values of testosterone in guinea pigs appeared after reaching puberty (day 60). This finding likely reflects the activity of the hypothalamic-pituitary-gonadal (HPG) axis. This axis in guinea pigs should behave similarly to the axis in humans, where it is suppressed in childhood and reactivated in puberty. In prepubertal children, the axis is inhibited by suppression of GnRH synthesis and pulsatile release [19]. The TT surge to reach puberty observed in castrated guinea pigs in our study could be due to the TT secreted by the adrenal glands, which we verified in prepubertal animals, in which 24.5% of testosterone was extragonadal (probably of adrenal origin) during prepuberty (based on comparison between castrated guinea and uncastrated guinea pigs), while 36% of testosterone was extragonadal at pubertal age (day 65). This percentage fell to 12% by day 80, which can be considered adulthood.

In the evaluation on day 80 (post-pubertal stage), the TT showed a drop in two experimental groups (Table 2; Fig. 2), with a significant difference between the groups ($P < 0.05$). Previous work [6] showed a TT drop from 6.1 ± 8.0 ng/mL on day 60 to 4.8 ± 8.0 ng/mL on day 90. Although these values are higher than those found in the present study, we observed here a similar pattern of TT secretion after puberty in our guinea pigs. Another study [21] described how testosterone levels controlled release of GnRH by the hypothalamus and gonadotropin release by the pituitary via positive or negative feedback systems, depending on age.

Based on this study, the fact that the guinea pigs of our groups E1-G1 and E1-G2 reached the highest values at puberty, respectively 3.63 ± 0.31 and 1.15 ± 0.31 ng/mL, may have triggered a negative feedback loop that inhibited the production of GnRH by the hypothalamus, which in turn inhibited production of FSH and LH by the pituitary gland. Ultimately, this may have decreased testosterone production to respective levels of 3.01 ± 0.26 and 0.34 ± 0.26 ng/mL. We conclude, therefore, that testosterone in guinea pigs follows a release pattern similar to that described for other male mammals: levels are basal during infancy, they rise at the pubertal stage and remain elevated but submaximal during adulthood. In addition, our results provide the first evidence that in guinea pigs, approximately 75% of testosterone is produced by the testicles and 25% extragonadally. Finally, we observed a testosterone rise around late puberty that was independent of the testicles, whose explanation requires further investigation.

3.1.2. Assessment of DPS development

The length of the left and right penile spicules of groups E1-G1 (non-castrated guinea pigs) and E1-G2 (castrated on day 35) were similar on day 20 of age ($P > 0.05$; Table 3). The two groups of guinea pigs showed a sustained increase in the length of their spicules, in correlation with TT levels until day 35 (right spicule: $r = 0.83$, $P < 0.01$; left spicule: $r = 0.80$, $P < 0.01$); these correlations were observed in left and right spicules, without significant difference between the two groups E1-G1 and E1-G2 ($P > 0.05$, Table 3).

This anatomical development is similar to that described in rats [22], in which androgens are responsible for the development and orientation of the penile papillae. A study of Wistar rats [8] found that testosterone levels exerted a direct influence on the development of penile spicules.

Spicules presented atrophy after guinea pigs were castrated on

Table 2
Means and standard error of total testosterone (TT, ng/mL) with age in guinea pigs.

Group	Animal age				
	20 d	35 d	50 d	65 d	80 d
E1-G1 (non-castrated)	0.77 ± 0.17	1.43 ± 0.55	2.06 ± 0.33[a]	3.63 ± 0.31[a]	3.01 ± 0.26[a]
E1-G2 (castrated)	0.81 ± 0.17	1.04 ± 0.55	0.49 ± 0.33[b]	1.15 ± 0.31[b]	0.34 ± 0.26[b]

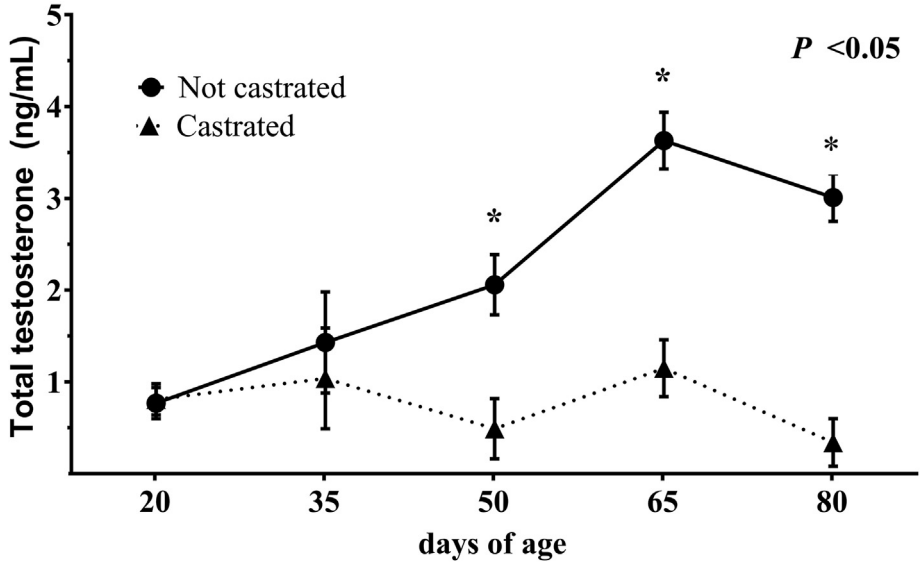

Fig. 2. Total testosterone assessment (TT) of non-castrated (E1-G1) and castrated (E1-G2) guinea pigs at ages of 20, 35, 50, 65 and 80 days.

day 35. Thus, the left spicule was reduced to an average length of 693.4 μm and right spicule to 677.3 μm during a period of 15 days after castration. However, the spicules of the E1-G1 group (non-castrated guinea pigs) increased in length during evaluations at days 50, 65, and 80 (Table 3). These results agree with findings that castration produced atrophy of penile spicules in rats (*Rattus norvegicus*) [23] and hamsters (*Mesocricetus auratus*) [9]. However, guinea pigs in group E1-G1 showed a small increase in the length of the left spicule (211 μm) and right spicule (266 μm) on day 65 of age, 30 days after castration. Nevertheless, on day 80, the length of the spicules of these animals decreased again, indicating that atrophy continued (Table 3). This slight growth of spicules at puberty (day 65) in the castrated group E1-G2 may be linked to the rise in extragonadal testosterone observed in Experiment 1, which also occurred on day 65 (late puberty). Based on these results, we conclude that the development of penile spicules in guinea pigs is androgen-dependent, similar to spicule development in rats [24].

3.2. Experiment 2

Serum TT levels before castration were similar between groups and similar to those observed in Experiment 1 (data not shown). After castration (day 35), guinea pigs showed significantly lower TT than the non-castrated group E2-G3 on day 50 ($P < 0.05$, Table 4), similar to what was observed in Experiment 1 and in previous work [7]. In the evaluation on day 65 (puberty), the four experimental groups, including castrated guinea pigs, showed an increase in TT levels before the first administration of exogenous testosterone (Table 4). This result is analogous to what was observed in Experiment 1.

The guinea pigs that received exogenous testosterone (E2-G1 and E2-G2) on day 65 (first dose) and day 80 (second dose) and that were evaluated at day 90 showed an increase in TT levels, which differed significantly ($P < 0.05$) from the levels in E2-G4 animals that had been castrated and did not receive exogenous testosterone. However, exogenous testosterone in E2-G1 and E2-G2 animals was not sufficient to reach the TT levels in non-castrated guinea pigs (Table 4). This may mean that the dose of exogenous testosterone was inadequate (125 μg in E2-G1 and 250 μg in E2-G2) or that the timing of administration (30 days after castration) or 15-day interval between the two doses was not optimal. Our protocol was adapted from a study in rats guinea [17], in which exogenous testosterone in oil was delivered subcutaneously at doses of 100–800 μg at one day after castration; that work reported that a dose of 100 μg was sufficient to restore the growth of spicules, and its effects did not significantly differ from those of 200 μg. In contrast, a study of TT in castrated hamsters (*Mesocricetus auratus*) [9] found lower, equal, or higher levels than in non-castrated animals, and the direction of the difference seemed to depend on the dose (25–1000 μg/day for 3 weeks) and formulation used. Therefore, it may be possible to achieve stable physiological levels of TT in guinea pigs through optimization of dose, frequency, and routes of administration of exogenous testosterone.

3.2.1. Assessment of DPS development

The left and right penile spicules of guinea pigs before castration on day 35 had a similar length in the four treatments ($P > 0.05$). After castration, in the evaluation at days 50 and 65, a notable decrease in the length of both left and right structures was observed in all castrated groups (Table 5).

Table 3
Means and standard error of penile spicule length (μm) in guinea pigs with age.

Animal age	20 d	35 d	50 d	65 d	80 d
Left spicule					
E1-G1	1586.9 ± 200.33	1609.0 ± 225.70	2085.5 ± 232.85[a]	2250.2 ± 62.89[a]	2724.7 ± 149.68[a]
E1-G2	1313.7 ± 200.33	1620.0 ± 225.70	926.6 ± 232.85[b]	1137.6 ± 62.89[b]	715.8 ± 149.68[b]
Right spicule					
E1-G1	1716.1 ± 167.22	1810.0 ± 290.75	2394.4 ± 193.76[a]	2817.9 ± 77.58[a]	3079.1 ± 33.55[a]
E1-G2	1687.4 ± 167.22	1693.5 ± 290.75	1016.2 ± 237.31[b]	1282.3 ± 77.58[b]	882.5 ± 41.08[b]

Table 4
Mean and standard error of testosterone levels (ng/mL) of guinea pigs with age, with or without administration of Testosterone Enanthate.

Group	Animal age			
	35 d	50 d	65 d	90 d
E2-G1 (castrated+125 μg ET)	1.43 ± 0.34	0.49 ± 0.34[b]	1.35 ± 0.34[b]	1.46 ± 0.34[b]
E2-G2 (castrated +250 μg ET)	1.15 ± 0.34	0.57 ± 0.34[b]	1.58 ± 0.34[b]	1.66 ± 0.34[b]
E2-G3 (non-castrated)	1.04 ± 0.34	2.06 ± 0.34[a]	4.03 ± 0.34[a]	3.41 ± 0.34[a]
E2-G4 (castrated)	1.01 ± 0.34	0.59 ± 0.34[b]	1.15 ± 0.34[b]	0.34 ± 0.34[c]

Castration in Sprague Dawley rats produces regression of penile spicules from day 9 onwards, with total atrophy on day 21 [25]. In that work, the atrophy of the penile spicules after castration on day 35 continued until day 90 (the end of the experiment) in the castrated group without exogenous testosterone. However, the spicules in our study never completely disappeared, similar to what was observed in hamsters [9]. This may be due to sustained extragonadal production of testosterone, as observed in the present work.

Assessment of DPS in groups E2-G1 and E2-G1 on day 90, after administration of two exogenous testosterone doses (on days 65 and 80), showed that the penile spicules had not only stopped atrophying but had resumed growth, becoming longer than spicules in the group E2-G4 (castrated guinea pigs without exogenous testosterone; $P < 0.05$). Nevertheless, this stimulus was not enough to reach the length observed in the animals of the group E2-G3 (non-castrated guinea pigs). This may mean that the doses of 125 μg in E2-G1 and 250 μg in E2-G2 were insufficient. Indeed, subcutaneous administration of androgens at 25–1000 μg/day to castrated hamsters restored the growth of penile spicules in a dose-dependent manner, with doses of at least 300 μg/day achieving the same length as in non-castrated controls [9].

Based on these results, we suggest that penile spicule development in guinea pigs is directly related to testosterone levels, and that eliminating the primary source of this hormone leads to spicule atrophy but not disappearance.

4. Conclusions

Testosterone levels in guinea pigs increase steadily until late puberty or hormonal maturity (day 65), then the levels decrease and stabilize. These variations are directly related to the development of penile spicules. Castrated animals still show 12–35% of the testosterone levels as normal animals, reflecting extragonadal production, which increases during puberty. Finally, we observed that exogenous administration of testosterone can halt and even reverse spicule atrophy in castrated males. Additional research is required to explain non-testicular testosterone production in guinea pig, including the oscillations in extragonadal levels and their guinea relevance to penile spicules.

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CRediT authorship contribution statement

Luis Ayala Guanga: Conceptualization, Methodology, Data curation, Writing - original draft, Supervision. **Susana Astiz:** Supervision, Conceptualization, Writing - review & editing. **Pedro Nieto Escandon:** Conceptualization, Investigation, Methodology. **Jorge Dutan Saramago:** Methodology, Writing - review & editing. **José Luis Pesantez-Pacheco:** Methodology, Writing - review & editing. **Cornelio Rosales Jaramillo:** Methodology, Writing - review & editing.

Declaration of competing interest

None.

E1-G1 = non-castrated guinea pigs; E1-G2 = guinea pigs castrated on day 35 of age. Superscripts a and b indicate statistically significant differences between experimental groups ($P < 0.05$).

E1-G1=non-castrated guinea pigs; E1-G2=guinea pigs castrated on day 35. Superscripts a and b indicate statistically significant differences between experimental groups ($P<0.05$).

E2-G1 = castrated guinea pigs + administration of 125 μg of Testosterone Enanthate; E2-G2 = castrated + administration of 250 μg of Testosterone Enanthate; E2-G3 = non-castrated; E2-G4 = castrated without exogenous testosterone. Exogenous testosterone (ET) was administered on days 65 and 80. Superscripts a, b and c indicate statistically significant differences between experimental groups ($P < 0.05$).

E2-G1 = castrated guinea pigs + administration of 125 μg of Testosterone Enanthate; E2-G2 = castrated + administration of 250 μg of Testosterone Enanthate; E2-G3 = non-castrated; E2-G4 = castrated without exogenous testosterone administration.

Table 5
Mean and standard error of penile spicule length (μm) in guinea pigs with age, with or without administration of Testosterone Enanthate.

Animal age	35 d	50 d	65 d	90 d
Left spicule				
E2-G1	1609.0 ± 176.26	1198.1 ± 135.79[a]	904.7 ± 101.81[a]	2545.8 ± 176.27[b]
E2-G2	1513.4 ± 176.26	1259.9 ± 135.79[a]	1142.2 ± 101.81[a]	2530.7 ± 176.27[b]
E2-G3	1620.0 ± 176.26	2394.4 ± 135.79[b]	2817.9 ± 101.81[b]	3079.1 ± 176.27[c]
E2-G4	1800.2 ± 176.26	1195.5 ± 135.79[a]	1090.0 ± 101.81[a]	1094.0 ± 176.27[a]
Right spicule				
E2-G1	1266.4 ± 175.89	1099.1 ± 155.39[a]	789.1 ± 73.36[a]	2214.1 ± 175.89[b]
E2-G2	1279.4 ± 175.89	1077.8 ± 155.39[a]	873.8 ± 73.36[a]	2242.2 ± 175.89[b]
E2-G3	1410.0 ± 175.89	2085.5 ± 155.39[b]	2250.2 ± 73.36[b]	2724.7 ± 175.89[c]
E2-G4	1208.0 ± 175.89	1044.8 ± 155.39[a]	999.4 ± 73.36[a]	876.0 ± 175.89[a]

Exogenous testosterone (ET) was administered on days 65 and 80. Superscripts a, b and c indicate statistically significant differences between experimental groups ($P < 0.05$).

Figure note: * indicates significant differences ($P < 0.05$).

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