Research Report

Serum testosterone concentrations in alpacas and llamas after human chorionic gonadotropin stimulation before and after castration

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Abstract

Veterinarians perform testosterone stimulation tests in animals suspected of bilateral cryptorchidism and/or incomplete castration. Period for circulating testosterone to become undetectable after castration varies among species. To our knowledge, clearance of circulating testosterone in camelids after castration has not been examined. Testosterone stimulation with human chorionic gonadotropin (hCG) has been described in several species, including alpacas. However, there is limited information regarding responses to hCG in male llamas. Purpose was to measure serum testosterone concentrations [T] in llamas and alpacas after hCG stimulation before and after castration. We hypothesized that [T] significantly increases after hCG treatment and decreases rapidly after castration. Testosterone stimulation test (using hCG) was performed in mature male alpacas (n = 9) and llamas (n = 7) 1 day prior to and 1 day after castration. Blood samples were collected; 1 sample before and 3 samples (2, 4, and 8 hours) after hCG treatment. In llamas and alpacas [T] increased ($p < 0.05$) > 2 fold (from prehCG values) within 2 hours after hCG treatment. PrehCG [T] were higher ($p < 0.05$) in alpacas than llamas but did not differ significantly between them after hCG stimulation; 24 hours after castration [T] were < 0.05 ng/ml.

Keywords: Luteinizing hormone, radioimmunoassay, testes, testicular weight

Introduction

Veterinarians measure baseline testosterone concentrations and perform testosterone stimulation tests in male animals suspected of bilateral cryptorchidism and/or incomplete castration.[1] The interval for circulating testosterone to decrease to undetectable concentrations varies among species after castration. In horses, testosterone half-life is 1 hour; concentrations are similar to geldings at 24 hours after castration.[2] In cats, serum testosterone concentrations [T] decreased to 3-12% of precastration concentrations within 1 hour after castration; similar to castrated cats at 24 hours.[3] In rats, [T] decreased significantly at 12 hours after castration; however, were detectable until 72 hours after castration.[4] To our knowledge, in camelids, clearance of circulating testosterone after castration has not been reported.

Measuring baseline concentrations and changes to [T] after exogenous gonadotropin releasing hormone (GnRH) or a GnRH analogue (e.g. fertirelin acetate) treatment (induces secretion of endogenous luteinizing hormone [LH])[5] is diagnostically useful. Binding of LH to receptors in testicular interstitial (Leydig) cells stimulates endogenous testosterone secretion.[6] Another method is human chorionic gonadotropin (hCG) treatment and hCG binds directly to testicular LH receptors. Testicular interstitial cells respond to intravenous hCG treatment with immediate cyclic adenosine monophosphate (cAMP) production and rapid increase in testosterone secretion.[7] Testosterone stimulation with hCG has been described in several species (Table 1). However, information (response to testosterone stimulation with hCG) in llamas is limited to small sample size (intact: n =1; cryptorchid: n = 1; castrated: n = 1).[8]

Purpose was to measure [T] in llamas and alpacas after hCG stimulation before and after castration. We hypothesized that [T] significantly increases after hCG treatment and is undetectable within 24 hours after castration, and has diagnostic value

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Table 1. Review of testosterone stimulation results after hCG treatment in mammals

Species	Dose	Results
Alpaca	3,000 IU	[T] increased 2-4 fold 2 hours after IV hCG injection[10]
Cat	500 IU	[T] increased 7-5 fold in 0.5 and 2 hours after IV hCG treatment, respectively[18]
Cow	10,000 IU	[T] increased 4-5 fold in 5 days after IM hCG treatment[19]
Dog	67.56 IU/kg	[T] increased 2-3 fold in 0.5 and 2 hours after SC hCG treatment, respectively[20]
Dog	35 IU/kg	[T] increased 2 fold in 6 hours after IM hCG treatment, respectively[21]
Dog	150 IU	[T] increased 2 fold in 3 hours after IM hCG treatment[22]
Dog	25 IU/kg	[T] increased 2 fold in 1 hour after hCG SC treatment[23]
Horse	5,000 IU	[T] increased 2-5 fold in 1 and 12 hours after IV hCG treatment, respectively[2]
Horse	10,000 IU	[T] increased 2-4 fold in 1 and 24 hours after IV hCG treatment, respectively[1]
Pig	3,000 IU	[T] increased 10-20 fold in 1 and 28 hours after IV hCG treatment, respectively[24]
Rat	25 IU/kg	[T] increased 7 fold in 1 hour after hCG treatment[23]
Sheep	1,000 IU	[T] increased 2-8 fold in 0.5 and 3 hours after IV hCG treatment, respectively[25]

IU: international units; IM: intramuscular; IV: intravenous; SC: subcutaneous; [T]: serum testosterone concentrations

for detecting retained testicular tissue after castration or cryptorchidism.

Materials and methods

Mature (6.1 ± 2.9 years; range: 2-10 years) intact male alpacas (n = 9) and llamas (n = 7) were used. Animal experiments were approved by Oregon State University Institutional Animal Care and Use Committee (Protocol #3293).

One day prior to and 1 day after castration, testosterone stimulation test (using hCG) was performed. Jugular blood samples were collected prior to intravenous treatment of hCG (2,500 IU [alpaca], 5,000 IU [llama], Chorulon®, Intervet, Inc./Merck Animal Health, Rahway, NJ) and 2, 4, and 8 hours after hCG treatment. Llama hCG dose was based on a dose used for testosterone stimulation test in a cryptorchid llama.[9] Alpaca hCG dose was half of llama dose (alpaca body weight [70.8 ± 12.0 kg; range: 50.0-88.6 kg] was about half of llama body weight [152.6 ± 30.4 kg; range: 104.5-195.4 kg]) and also was similar to a dose used.[10]

Next day, males were castrated under intravenous general anesthesia (Telazol®, Zoetis, Parsippany, NJ) using closed castration technique. Scrotal incision was made over each testis and fascia surrounding testis was stripped; spermatic cord was crushed using a modified White's emasculator. Vaginal tunic and epididymides were removed (Figure 1) and testes were weighed. Skin incisions were allowed to heal by second intention.

Cornell University Animal Health and Diagnostic Center was used to measure [T] utilizing a double antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Samples were tested within 1 assay and intra-assay coefficients of variability were < 10%. Assay sensitivity was 0.1 ng/ml and the lowest detectable concentration was 0.05 ng/ml.

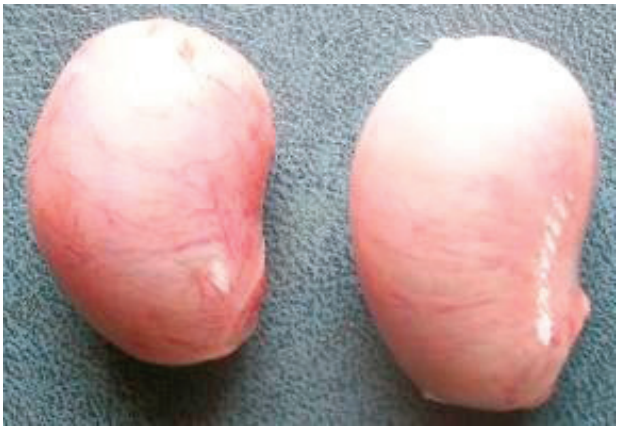

Figure 1. Pair of alpaca testes (after vaginal tunic and epididymides removal) prepared for weighing

Changes in mean ± SEM [T] after hCG treatment were analyzed using repeated measures analysis of variance and Dunnett's post hoc analysis (GraphPad Prism, San Diego, CA) and compared between species using Student's t-test (Microsoft Excel, Redmond, WA). Simple linear regression was used to compare [T] to testicular weight (Microsoft Excel; GraphPad Prism). Data are presented as mean ± SEM. Significance was defined as $p < 0.05$.

Results

Testosterone responses to hCG treatment are summarized (Table 2) and illustrated (Figure 2). In llamas and alpacas, [T] increased ($p < 0.05$) > 2 fold from prehCG values within 2 hours after hCG treatment and remained elevated until 8 hours. There were no significant differences in [T] among 2, 4, and 8 hours after hCG treatment. PrehCG [T] were higher ($p < 0.05$) in alpacas than llamas but did not differ significantly after hCG stimulation; 24 hours after castration [T] was < 0.05 ng/ml. Stimulation with hCG also resulted in

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Table 2. Testicular weights (TW) and serum testosterone concentrations ([T]) relative to the timing of human chorionic gonadotropin (hCG) treatment in alpacas and llamas, minimum (in **green** font) and maximum (in **red** font) values are identified in each column for alpacas and llamas; *differed ($p < 0.05$) from prehCG value

Alpaca	TW	PrehCG [T]	2 hours posthCG [T]	4 hours posthCG [T]	8 hours posthCG [T]
1	23.5 grams	3.23 ng/ml	10.32 ng/ml	15.53 ng/ml	20.37 ng/ml
2	22.6 grams	7.72 ng/ml	14.30 ng/ml	14.21 ng/ml	18.58 ng/ml
3	22.0 grams	3.62 ng/ml	5.98 ng/ml	10.43 ng/ml	8.53 ng/ml
4	28.8 grams	4.22 ng/ml	9.89 ng/ml	9.74 ng/ml	13.25 ng/ml
5	28.1 grams	4.44 ng/ml	7.15 ng/ml	6.33 ng/ml	6.96 ng/ml
6	17.9 grams	7.14 ng/ml	10.65 ng/ml	16.21 ng/ml	19.23 ng/ml
7	**14.7 grams**	**0.67 ng/ml**	**3.81 ng/ml**	**6.26 ng/ml**	**4.71 ng/ml**
8	28.9 grams	**9.12 ng/ml**	17.66 ng/ml	**20.89 ng/ml**	24.91 ng/ml
9	**36.2 grams**	5.60 ng/ml	**18.48 ng/ml**	19.83 ng/ml	**24.94 ng/ml**
Mean ± SEM	**24.7 ± 1.3 grams**	**5.1 ± 0.9 ng/ml**	**10.9 ± 1.7 ng/ml***	**13.3 ± 1.8 ng/ml***	**14.6 ± 2.5 ng/ml**
Llama	**TW**	**PrehCG [T]**	**2 hours posthCG [T]**	**4 hours posthCG [T]**	**8 hours posthCG [T]**
10	35.9 grams	1.5 ng/ml	5.0 ng/ml	5.3 ng/ml	5.71 ng/ml
11	30.1 grams	0.9 ng/ml	6.9 ng/ml	8.5 ng/ml	9.73 ng/ml
12	38.5 grams	2.1 ng/ml	**10.1 ng/ml**	**12.3 ng/ml**	**15.66 ng/ml**
13	32.7 grams	0.6 ng/ml	5.1 ng/ml	6.1 ng/ml	7.51 ng/ml
14	22.0 grams	0.5 ng/ml	6.1 ng/ml	9.4 ng/ml	10.42 ng/ml
15	**42.0 grams**	**3.0 ng/ml**	5.5 ng/ml	5.6 ng/ml	5.62 ng/ml
16	**9.9 grams**	**0.2 ng/ml**	**2.7 ng/ml**	**3.3 ng/ml**	**3.58 ng/ml**
Mean ± SEM	**30.2 ± 1.8 grams**	**1.3 ± 0.4 ng/ml**	**5.9 ± 0.8 ng/ml***	**7.2 ± 1.1 ng/ml***	**8.7 ± 1.5 ng/ml**

[T] < 0.05 ng/ml at 2, 4, and 8 hours in llamas and alpacas after castration (Table 3).

PrehCG [T] were higher ($p < 0.05$) in alpacas than llamas but did not differ significantly after hCG stimulation; 24 hours after castration [T] was < 0.05 ng/ml. Relationships between testicular weight and [T] are provided (Figure 3). There was moderate relationship ($R^2 = 0.69$, $p = 0.02$) between testicular weight and [T] prior to hCG treatment in llamas, but not in alpacas ($R^2 = 0.13$, $p = 0.35$). There was a weak relationship between testicular weight and [T] 2 hours after hCG treatment in alpacas ($R^2 = 0.46$, $p = 0.04$) but not in llamas ($R^2 = 0.33$, $p = 0.17$). There was no relationship between testicular weight and [T] at 4 hours (llamas: $R^2 = 0.12$, $p = 0.44$; alpacas: $R^2 = 0.19$, $p = 0.24$) and 8 hours (llamas: $R^2 = 0.12$, $p = 0.45$; alpacas: $R^2 = 0.25$, $p = 0.17$) after hCG treatment.

Discussion

We demonstrated that testosterone stimulation test is a diagnostic tool to detect testicular tissue in llamas and alpacas and documented that within 24 hours after testicular tissue removal, testosterone concentrations were undetectable; similar to horses and cats.[2,3] However, anecdotal evidence (https://open.lib.umn.edu/largeanimalsurgery/chapter/camelid-castration/) in noncamelid species suggested that males may be fertile and exhibit reproductive behaviors up to 6 weeks after castration; these parameters were not evaluated in the present study.

Endogenous testosterone secretion can be stimulated by GnRH or hCG; GnRH stimulates endogenous luteinizing hormone release from anterior pituitary that stimulates testosterone secretion from testicular Leydig cells, whereas hCG directly stimulates testosterone release from Leydig cells. For this reason, hCG is recommended over GnRH for evaluating testicular function.[11] In horses, [T] increased nearly 5 fold within 1 hour after hCG treatment.[2] In alpacas [T] increased 2-4 fold at 2 hours after hCG treatment.[10] Similar increases in [T] were not observed in our study in llamas and alpacas at 2, 4, and 8 hours after hCG treatment. However, after castration, hCG treatment did not increase [T] in llamas or alpacas, as in humans.[12]

Increased testicular weight may be correlated with increased steroidogenic cells (e.g. Leydig cells); larger mass of steroidogenic tissue increased Leydig cell sensitivity and resulted in higher [T].[13] This was supported by a study[10] in alpacas that demonstrated association ($R^2 = 0.72$; $p < 0.001$) between testicular weight and [T] 2 hours after hCG treatment. However, in stallions, there was no relationship between testicular weight and [T] before or after hCG treatment.[14] In our study, there was only moderate (significant) relationship ($R^2 = 0.69$; $p = 0.02$) between testicular weight and [T] prior to hCG treatment in llamas, but not in alpacas ($R^2 = 0.13$). There was also moderate (significant) relationship ($R^2 = 0.46$; $p = 0.04$)

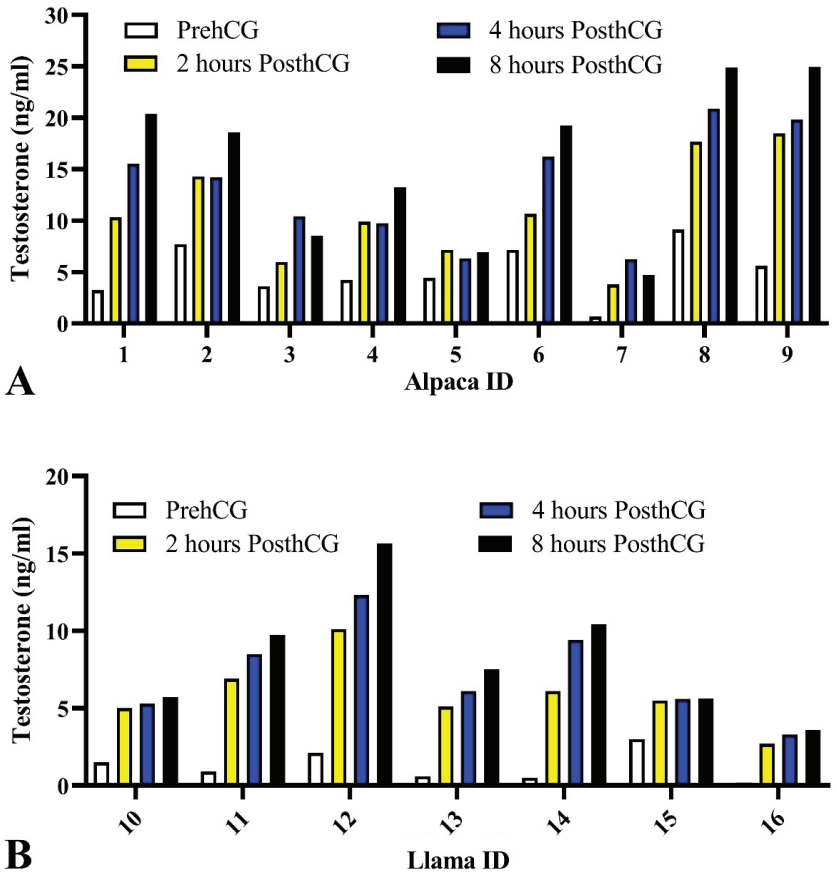

Figure 2. Serum testosterone concentrations in alpacas (A) and llamas (B) before and 2, 4, and 8 hours after human chorionic gonadotropin (hCG) treatment

Table 3. Serum testosterone concentrations ([T]) relative to human chorionic gonadotropin (hCG) treatment in alpacas and llamas 1 day after castration; lowest limit of detection for the testosterone assay was 0.05 ng/ml

Alpaca	PrehCG [T]	2 hours posthCG [T]	4 hours posthCG [T]	8 hours posthCG [T]
1	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
2	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
3	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
4	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
5	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
6	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
7	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
8	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
9	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
Llama	**PrehCG [T]**	**2 hours posthCG [T]**	**4 hours posthCG [T]**	**8 hours posthCG [T]**
10	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
11	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
12	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
13	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
14	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
15	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml
16	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml	< 0.05 ng/ml

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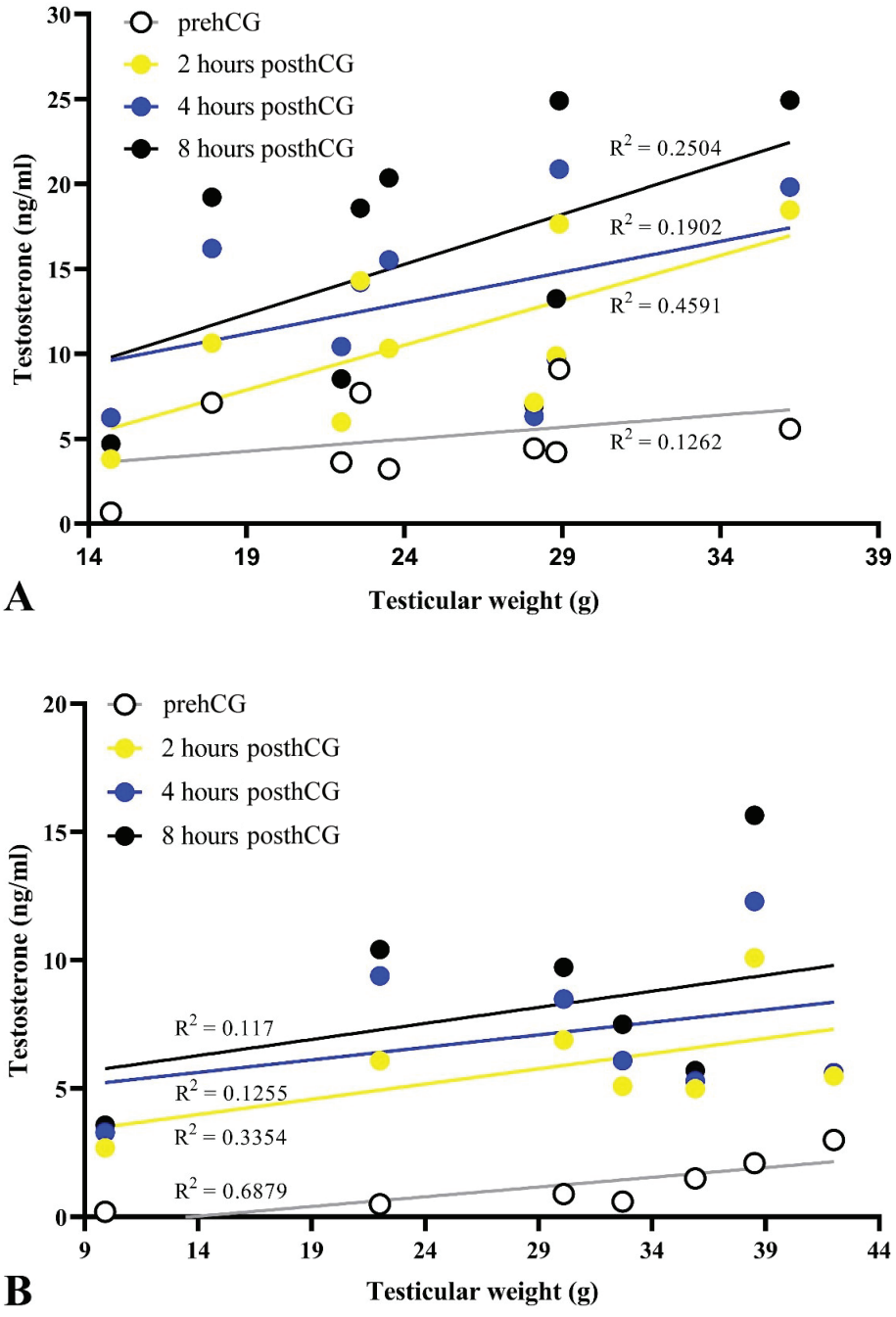

Figure 3. Relationships between serum testicular concentrations and testicular weight in alpacas (A) and llamas (B) before and 2, 4, and 8 hours after human chorionic gonadotropin (hCG) treatment. Only relationships before hCG treatment in llamas and 2 hours after hCG in alpacas were different ($p < 0.05$)

between testicular weight and [T] 2 hours after hCG in alpacas but not in llamas ($R^2 = 0.33$; $p = 0.17$). There was no significant relationship between testicular weight and [T] at 4 and 8 hours after hCG treatment in llamas and alpacas ($R^2 = 0.12$-0.25). These findings suggested that Leydig cell responsiveness to hCG is not related to testicular weight.

A potential limitation of the current study was that antiMüllerian hormone (AMH) concentrations were not measured. A recent paper[15] stressed the importance of examining AMH concentrations in male alpacas. In addition to measuring testosterone concentration after hCG stimulation, AMH concentrations can provide evidence for cryptorchid testis. However, testosterone stimulation with hCG remains the gold standard for determining potential cases of cryptorchidism.[16,17]

Conclusion

In summary, hCG treatment in mature intact male llamas and alpacas increased [T] by 2 fold within 2 hours and remained

elevated for at least 8 hours. In addition, [T] was below detectable limits within 24 hours after castration.

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Conflict of interest

None to report.

Author contributions

MK conceived the idea, garnered funding and performed experiments, AM wrote substantial part of the manuscript, MK, AM, and RH revised it, and all authors approved submission.

References

1. Arighi M, Bosu WTK: Comparison of hormonal methods for diagnosis of cryptorchidism in horses. J Equine Vet Sci 1989;9:20-26. doi: 10.1016/s0737-0806(89)80112-1
2. Esteller-Vico A, Ball BA, Bridges JW, et al: Changes in circulating concentrations of testosterone and estrone sulfate after human chorionic gonadotropin administration and subsequent to castration of 2-year-old stallions. Anim Reprod Sci 2021;225:106670. doi: 10.1016/j.anireprosci.2020.106670
3. Johnstone IP, Bancroft BJ, McFarlane JR: Testosterone and androstenedione profiles in the blood of domestic tom-cats. Anim Reprod Sci 1984;7:363-375. doi: 10.1016/0378-4320(84)90021-6
4. Kniewald Z, Zanisi M, Martini L: Studies on the biosynthesis of testosterone in the rat. Acta Endocrinol (Copenh) 1971;68: 614-624. doi: 10.1530/acta.0.0680614
5. Sakase M, Weerakoon WWPN, Hannan MA, et al: LH and testosterone secretions in response to GnRH challenge in pubertal Japanese black beef bulls with normal and abnormal semen. J Vet Med Sci 2018;80:1829-1833. doi: 10.1292/jvms.18-0151
6. Dehejia A, Nozu K, Catt KJ, et al: Luteinizing hormone receptors and gonadotropic activation of purified rat Leydig cells. J Biol Chem 1982;257:13781-13786. doi: 10.1016/S0021-9258(18) 33517-8
7. Haour F, Dray F, Mather JP: In vivo and in vitro response of Leydig cells to acute stimulation by hCG. Ann N Y Acad Sci 1982;383: 231-248. doi: 10.1111/j.1749-6632.1982.tb23171.x
8. Bravo WP, Johnson LW: Reproductive physiology of the male camelid. Vet Clin North Am Food Anim Pract 1994;10:259-264. doi: 10.1016/s0749-0720(15)30560-0
9. Perkins NR, Frazer GS, Hull BL: Endocrine diagnosis of cryptorchidism in a llama. Aust Vet J 1996;74:275-277. doi: 10.1111/j.1751-0813.1996.tb13773.x
10. El Zawam A, Tibary A, Patino C: Basal levels and hCG response of serum testosterone and estrogen in male alpacas. Front Vet Sci 2020;7:595856. doi: 10.3389/fvets.2020.595856
11. Roser J: Equine profiles in fertile, subfertile, and infertile stallions: testicular response to human chorionic gonadotropin in infertile stallions. Bio Reprod Monogr 1995;1:661-669. doi: 10.1093/biolreprod/52.monograph_series1.661
12. Bergman B, Damber JE, Tomić R: Effects of total and subcapsular orchidectomy on serum concentrations of testosterone and pituitary hormones in patients with carcinoma of the prostate. Urol Int 1982;37:139-144. doi: 10.1159/000280809
13. Allrich RD, Christenson RK, Ford JJ, et al: Pubertal development of the boar: testosterone, estradiol-17 beta, cortisol and LH concentrations before and after castration at various ages. J Anim Sci 1982;55:1139-1146. doi: 10.2527/jas1982.5551139x
14. Cox JE, Williams JH: Some aspects of the reproductive endocrinology of the stallion and cryptorchid. J Reprod Fert Suppl 1975;23:75-79.
15. Ciccarelli M, Tibary A, Campbell AJ, et al: Effect of age and castration on serum anti-Müllerian hormone concentration in male alpacas. Theriogenology 2018;105:174-177. doi: 10.1016/j.theriogenology.2017.09.032
16. Marzok M, Moustafa A, El-Habashi N, et al: Evaluation of modified in situ pinhole technique for castration in horses. Kafrelsheikh Vet Med J 2016;14:163-179. doi: 10.21608/kvmj.2016.108687
17. McEachern R, Houle AM, Garel L, et al: Lost and found testes: the importance of the hCG stimulation test and other testicular markers to confirm a surgical declaration of anorchia. Horm Res 2004;62:124-128. doi: 10.1159/000080018
18. Memon MA, Ganjam VK, Pavletic MM, et al: Use of human chorionic gonadotropin stimulation test to do detect a retained testis in a cat. J Am Vet Med Assoc 1992;201:1602. doi: 10.2460/javma.1992.201.10.1602
19. Murase T, Okuda K, Sato K: Assessment of bull fertility using a mucus penetration test and a human chorionic gonadotrophin stimulation test. Theriogenology 1990;34:801-812. doi: 10.1016/0093-691X(90)90552-5
20. Santana M, Batista M, Alamo D, et al: Influence of sexual stimulation and the administration of human chorionic gonadotrophin on plasma testosterone levels in dogs. Reprod Domest Anim 2012;47:43-46. doi: 10.1111/j.1439-0531.2011.01923.x
21. Mialot JP, Thibier M, Toublanc JE, et al: Plasma concentration of luteinizing hormone, testosterone, dehydroepiandrosterone, androstenedione between birth and one year in the male dog: longitudinal study and hCG stimulation. Andrologia 1987;20: 145-154. doi: 10.1111/j.1439-0272.1988.tb00678.x
22. Sundby A, Ulstein T: Plasma concentrations of testosterone in the male dog and plasma testosterone profile following single intramuscular injection of HCG. Acta Vet Scand 1981;22:409-416. doi: 10.1186/BF03548666
23. Tremblay Y, Belanger A: Changes in plasma steroid levels after single administration of hCG or LNRH agonist analogue in dog and rat. J Steroid Biochem 1985;22:315-320. doi: 10.1016/0022-4731(85)90432-7
24. Andresen O: 5alpha-androstenone in peripheral plasma of pigs, diurnal variation in boars, effects of intravenous HCG administration and castration. Acta Endocrinol (Copenh) 1975;78:385-391. doi: 10.1530/acta.0.0780385
25. Rhodes RC, Fleming MW: Unilateral orchidectomy of ram lambs: acute, chronic and hCG-stimulated androgen levels. Comp Biochem Physiol 1987;87:627-630. doi: 10.1016/0300-9629(87)90372-0