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Research Article

Serum and Testicular Testosterone Levels of Ram Lamb during Puberty

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Abstract | This study was done in order to investigate the levels of serum and testicular testosterone during puberty age in sheep. Twelve lambs aged at 3 months and two mature rams aged at 3 years were subject to this study. Serum testosterone was taken every two weeks for six months from all animals starting from November 2017 to April 2018. Castration was done to mature males at the end of this study. Castration for young lambs was done for twoof them every month. Serum and testicular testosterone were analyzed with commercial gamma counter kits. Results showed that there were a significant differences (P<0.05) between mature rams and lambs under 6 months age. The testicular testosterone showed significant differences (P<0.05) between mature rams and lambs under 5 months of age, with significant (P<0.01) correlation with hormonal level and ages. It could be concluded that the serum and the testicular testosterone could be used as an indicator for puberty age with more accuracy for testicular level in sheep.

Keywords | Puberty, Ram lamb, Testosterone, Testicular, Serum.

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INTRODUCTION

The testosterone is a steroid hormone contain 19 carbon atom it execrates mainly from testes and produces from cholesterol in Leydig cell, and also it produces small amount in adrenal cortex (Ganong, 2003). The testosterone execrate under effect of ICSH hormone which stimulate by Leydig cells to increase cAMP then rise the convert cholesterol esterase into cholesterol and then into pregnenolone (Pineda and Dooley, 2003). About 98% of testosterone transported into target cells through blood as β-steroid binding globulin, the remnant 2% still free and enter the target cells, where then converted into active dihydrotestosterone which act on nucleus receptors (Al-kawmani et al., 2014). During fetal stage the testes excrete testosterone that is responsible for the development of Wolffian duct, and this fetal testosterone responsible of differentiation of male and testes descend into scrotal sac before parturition (Ferra et al., 2010). The testes testosterone release especially during early sexual fetal differentiation, through production of HCG from placenta and ICSH from pituitary (Gromoll et al., 2000). The testes after sexual differentiation release androgens especially 5α dihydrtestosterone that lead to development of male organs (Gromoll et al., 2000). The fetal testosterone is also produced from adrenal cortex, placenta which produces estrogen, the testosterone is a middle product between progesterone and estrogen, and there was a high level of testosterone and androstendione in sheep fetal blood before lambing and increase during parturition (Pineda and Dooley, 2003). The testis of sheep release testosterone that elevate with increasing of testicular weight and age, until the puberty and maturity age (Khalifa et al., 2013). Testosterone is essential for the expansion and upkeep of sexual behavior in rams. The sexual efficiency increases withelevated levels of testosterone during puberty (Perkins and Roselli, 2007). Majority of testosterone (which is produced by testes) is responsible for the development of primary and secondary sexual characteristics, sperm production, and the regulation of sexual behavior (Pelletier et al., 2003). In the same side testosterone was low at 30-180 days of age, and rise to 8 ng/ml at 200 days of age. After that, there was an

increasing of its level in blood to reach 2.7-5.1 ng/ml at 41 weeks of age, this elevation of testosterone hormone with advancing of age considered as indicator for the increasing of testicular activity (Rajak et al., 2014). The increasing of testicular weight due to increasing of absolute weight of cells that mostly converted into specialized interstitial cells at 6 months of age, which characterized with large weight than unspecialized cells (Myers et al., 2005). The increase of testosterone hormone was associated with increasing of testicular volume and its diameters, and the development and growth of epididymis depend on the growth of testes and testosterone level which affect as dihydrotestosterone (DHT) in epididymis growth (Castro et al., 2002). The rapid growth of epididymi sis related to increasing of the testosterone receptors, this hormone lead to improve the action of epididymis and rising of accessory glands leading to increases the ejaculation volume (Boukenaoui et al., 2012). After birth, the testes produce androgen under control of ICSH increases steroid and cholesterol enzymes activity which then regulate the release of GnRH, SSH, Prolactin, PGF2α, growth hormone and Insulin. The testosterone decreases after birth due to its decreasing from fetal interstitial cells that converted into postnatal generation of Leydig cells, at maturity age there was increasing of releasing activity of this cells that increase testosterone level which accompanied with ICSH secretion (Ferra et al., 2010). Starting concentrations of testosterone arerequired for the gaining and exhibit of adult sexual behavior. Early exhibit to ewes enhances ram sexual performance, but cannot prevent some rams from displaying male-oriented sexual partner preferences (Perkins and Roselli, 2007).

MATERIALS AND METHODS

Twelve male lambs of Awassi male lambs aged at 3 months old (determined by teeth formula in accordance with to Dyce and Sack (2010) and of 10-12 kg body weight where subjected to this study. All animals kept under similar conditions. After weaning they were separated from their mothers and fed with two periods of grazing. The first one started from sunrise to midnoon, then they took 2 hours of rest then the second grazing period started into sunset time, the water and minerals blocks approved ad libitum. The medical vaccination and treatment where given during the study period (November 2017 to April 2018). All animals were kept in Jeballa town in Babel province southern to Baghdad, Iraq. Another two mature Awassi rams and ewes aged 3 years old were kept as control with these lambs as post-pubertal ages.

All animals were clinically examinedand that included: general evaluation(body condition inspection, detection of hereditary defects, respiratory, circulatory, digestive and musculoskeletal systems) and special examination of genitalia (inspection and palpation of the scrotum, testes, epididymis, spermatic cords, prepuce and penis). This was done according to Andrade et al. (2014).

Ten ml of blood samples were collected every two weeks from jugular vein using 20-gauge syringe from all rams and lambs. Serum was separated by centrifugation at 3000 rpm for 10 minutes, then the serum was collected and stored at -20 C°. The castration was done every month on two lambs, and at the study on the mature rams. The testicular testosterone analysis on the testicular tissue after castration was done by homogenizing and mashing it with mixer. Fluid was extracted with ether (Weldall) and stored at -20C° until analysis.This was done according to the method of Castro et al. (2002) with some modification. The testosterone was evaluated using Roche Cobas (Roche Company) commercial gamma counter kit.

Statistical Analysis

Statistical analysis was carried out to study the differences between means. ANOVA test was performed. Duncan multiple range testswere used to compare different means of studied parameters. Correlations were examined for studied lambs and rams. This was done according to Al-Mohammed et al. (1986).

Table 1: The serum testosterone (ng/dL) and testicular testosterone (ng/dL) in pre-pubertal lambs and mature rams.

Ages	Serum testosterone*	Testicular testosterone*
3 months	6.36±0.9 c	24.6±1.1 b
4 months	8.53±0.8 c	35.1±0.9 b
5 months	13.7±0.8 c	428±40 b
6 months	42.3±0.6 bc	1321.4±120 ab
7 months	104.8±1 abc	5369.9±303 ab
8 months	131.9±2.5 ab	6758.5±419 ab
9 months	138.5±2.6 ab	7886±916 ab
Mature	159.07±3.5a	9093.1±190.1 a
Correlations	+0.184	+0.69**

The numbers represent mean ± standard error.
The similar small letters represent no significant differences.
The different small letters represent significant differences at level of *(P<0.05) or **(P<0.01).

RESULTS

The study demonstrated that there were significant differences (P<0.05) in serum testosterone of mature aged and those under 7 months of age, and significant differences were recorded between 5 and 8 months lambs (Table 1). There was a positive correlation 0.184 noticed with advance of age in serum hormone level (Table 1). In addition to that there were significant differences (P<0.05) between

mature rams testicular testosterone and lambs under 6 months of age, and no other significance was noticed (Table 1). There was a significant (P<0.01) correlation +0.69 between age increase and testicular testosterone (Table 1).

DISCUSSIONS

The recent results indicated that the 8 and 9 months aged and mature rams, showed significant differences compared with those lambs aged less than 6 months. This result was agreement with many previous studies (Mahmoud, 2002; Elmaz et al., 2007; Nazari-Zenouz et al., 2016). Also the mean of hormonal levels was fit with those which recorded by (Mahmoud, 2002) in pubertal lambs, and lower than those which recorded by (Elmaz et al., 2007; Kishk, 2008; Benia et al., 2013; Belkadi et al., 2017) in adult and young animals. The mean serum testosterone level appears swing sharply between 240 and 320 days of age with significant differences at (P<0.05) and the highest testosterone levels were recorded at 260 and 300 days of age by (Elmaz et al., 2007). Onset of puberty was observed to be established at 8–9 months of age when the testosterone plasma level reached 2 ng/ml which involved to the increase in testicular testosterone secreting (Nazari-Zenouz et al., 2016). This is may be to the lack of Leydig cells between small and large animals or decreasing of it after birth due to decreasing of interstitial cells that converted into postnatal generation of Leydig cells.

The level of testosterone in the blood circulation of rams varies by breed, age, nutrition, season, and manifestations of estrus in ewes (Kridli et al., 2006). Although there was a structural and functional combination of testisrelease of testosterone and sperm so that causes the testosterone-dependent quality of spermatogenesis (Masanyi et al., 2000). There were variable characteristic of testosterone levels of age between 2 and 14 months rams, with the lowest values recorded between theages of 80 and 100 days and the highest values was recorded between the ages of 260 and 300 days (Elmaz et al., 2007). This is may be due to different in cells number and seasons. Moreover, an increase in the testosterone level with age aswell as vibrates in the determined values (Ungerfeld and Gonzalez-Pensado, 2008). Preston et al. (2012) reported that the production of testosterone changed during the life of rams, with increasing levels of hormones from birth until they reach full sexual maturity and a decrease thereafter. Higher testosterone concentration in the group having scrotal circumference>25cm may be the main factor affecting testes measurement, and better libido compared to rams having scrotal circumference≤25cm (Charles et al., 2002; Stellflug et al., 2004) indicating the important role of testosterone hormone for regulating sexual behavior. Increase in LH and testosterone levels in peripheral blood leads to string sexual behavior (Mahmoud, 2002). Concentrations of these hormones were found to be greater in sexuallyactive than in sexually inactive rams when exposed to estrus ewes (Stellflug et al., 2004).

CONCLUSIONS

The testosterone hormone could be used as an indicator for detecting puberty age in lambs, and the testicular level show more accuracy than serum's level.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHORS CONTRIBUTION

Both authors contributed equally.

REFERENCES

- Al-Kawmani AA, Alfuraiji MM, Abou-Tarboush FM, Alodan MA, Farah MA (2014). Developmental changes in testicular interstitium in the Najdi Ram Lambs. Saudi J. Biolog. Sci. 21: 133-137. https://doi.org/10.1016/j.sjbs.2013.09.001
- Al-Mohammed NT, Al-Rawi KM, Younis MA, Al-Morani WK (1986). Principles of Statistics. Book House for Printing and Publishing, University of Al-Mosel.
- Andrade AKG, Soares AT, Freitas FF, Silva SV, Pena-Alfaro CE, Batista AM, Guerra MMP (2014). Testicular and epididymal ultrasonography in Santa Ines lambs raised in Brazil. Anim. Reprod. 11 (2): 110-118.
- Belkadi S, Safsaf B, Heleili N, Tlidjane M, Belkacem L, Oucheriah Y (2017). Seasonal influence on sperm parameters, scrotal measurements, and serum testosterone in Ouled Djellal breed rams in Algeria. Vet. World. 10 (12): 1486-1492. https://doi.org/10.14202/vetworld.2017.1486-1492.
- Benia AR, Taibi K, Ait-Amrane A, Belhamiti T, Hammoudi SM, Kaidi R (2013). Study of seasonal sexual activity variations in Algerian rams: Sexual behaviour, testosterone concentration control and environmental factors. African J. Biotechnol. 12 (41): 6042-6048. https://doi.org/10.5897/AJB2013.12172
- Boukenaoui N, Moudilou E, Chevalier C, Amirat Z, Exbrayat JM, Khammar F (2012). Postnatal changes in testicular development and androgen receptors immunolocalization in D'Man ram lambs. Folia Histochmica et Cytobiologica. 50 (1): 38–45. https://doi.org/10.5603/FHC.2012.0005
- Castro CS, Berndtson WS, Cardoso FM (2002). Plasma and testicular testosterone levels, volume density and number

of Leydig cells and spermatogenic efficiency of rabbits. Brazilian J. Med. Biolog. Res. 35: 493-498. https://doi.org/10.1590/S0100-879X2002000400014

- Charles ER, Stormshak F, Stellflug JN, Resko JA (2002). Relationship of serum testosterone concentrations to mate preferences in rams. Biol. Reprod. 67: 263–268. https://doi.org/10.1095/biolreprod67.1.263
- Dyce KM, Sack WO (2010). Text Book of Veterinary Anatomy. 4th ed. Edinburgh, Scotland. p: 100.
- Elmaz O, Cirit U, Demir H (2007). Relationship of testicular development with age, body weight, semen characteristics and testosterone in Kivircik ram lambs. South African J. Anim. Sci. 37: 269–274. https://doi.org/10.4314/sajas.v37i4.4100
- Ferra JC, Cieslak S, Filho RS, McManus C, Martins CF, Sereno JRB (2010). Weight and age at puberty and their correlations with morphometric measurements in crossbred breed Suffolk ewe lambs. Revista Brasil. Zoot. 39 (1): 134-141. https://doi.org/10.1590/S1516-35982010000100018
- Ganong WF (2003). Review of Medical Physiology. 21st ed. Lange Medical Books-McGraw Hill, Los Altos, California. Pp: 364-366.
- Gromoll J, Eiholzer U, Nieschlage E (2000). Male hypogonadism caused by homozygous deletion of axon10 of the leuteinizing hormone (LH) receptor; differentia action of HCG and LH. J. Clin. Endocrinol. Metabol. 85: 2281-2286. https://doi.org/10.1210/jcem.85.6.6636
- Khalifa EI, Ahmed ME, Hafez YH, El-Zolaky OA, Bahera KM, Abido AA (2013). Age at puberty and fertility of Rahmani sheep fed on biological inoculated corn silage. Annals Agric. Sci. 58 (2): 163–172. https://doi.org/10.1016/j.aoas.2013.07.003
- Kishk HW (2008). Interrelationship between ram plasma testosterone level and some semen characteristics. Slovak J. Anim. Sci. 41: 67–71.
- Kridli TR, Abdullah YA, Shaker MM, Al-Momani QA (2006). Age at puberty and some biological parameters of Awassi and its first crosses with Charollais and Romanov rams. Italian J. Anim. Sci. 5: 193–202. https://doi.org/10.4081/ijas.2006.193
- Mahmoud GBA (2002). Some reproductive characteristics in Saidi ram lambs as affected by feeding different levels of energy and protein. Master Science Thesis, Faculty of Agriculture, Assuit University, Assuit, Egypt.
- Masanyi P, Jancova A, Uhrin V (2000). Quantitative microscopic analysis of testes and epididymis in Apodemusflavicollis (Murridae). The 6th Internet World Congress for Biomedical Sciences, on Uropathology in Human and Experimental tissues Symposium. Presentation. No. 4.
- Myers M, Ebling FJ, Nwagwu M, Boulton R, Wadhwa K, Stewart J, Kerr JB (2005). Atypical development of Sertoli cells and impairment of spermatogenesis in the hypogonadal (hpg) mouse. J. Anat. 207 (6): 797-811. https://doi.org/10.1111/j.1469-7580.2005.00493.x
- Nazari-Zenouz F, Moghaddam G, Hamidiam G, Ashrafi J, Rafat SA, Qasemi-panahi B (2016). Postnatal testicular development and testosterone changes in Ghezel ram lambs. Small Rumin. Res. 141: 70-76. https://doi.org/10.1016/j.smallrumres.2016.07.001
- Pelletier F, Bauman J, Festa-Bianchet M (2003). Fecal testosterone in bighorn sheep (Ovis canadensis): behavioural and endocrine correlates. Canadian J. Zool. 81: 1678–1684 https://doi.org/10.1139/z03-156.
- Perkins A, Roselli CE (2007). The ram as a model for behavioral euroendocrinology. Hormon. Behav. 52: 70–77. https://doi.org/10.1016/j.yhbeh.2007.03.016
- Pineda MH, Dooley MP (2003). The biology of sex. In; McDonald's Veterinary Endocrinology and Reproduction. 5th ed. Low State Press, A Black-Well Publishing Company. Pp: 17-32.
- Preston TB, Stevenson RI, Lincoln AG, Monfort LS, Pilkington GJ, Wilson K (2012). Testes size, testosterone production and reproductive behaviour in a natural mammalian mating system. J. Anim. Ecol. 81: 296–305. https://doi.org/10.1111/j.1365-2656.2011.01907.x
- Rajak SK, Kumaresan A, Gaurav MK, Layek SS, Mohanty TK, Muhammad Aslam MK, Tripathi UK, Prasad S, De S (2014). Testicular Cell Indices and Peripheral Blood Testosterone Concentrations in Relation to Age and Semen Quality in Crossbred (Holstein Friesian×Tharparkar) Bulls. Asian-Australasian J. Anim. Sci. 27 (11): 1554-1561.
- Stellflug JN, Perkins A, LaVoie VA (2004). Testosterone and luteinizing hormone responses to naloxone help predict sexual performance in rams. J. Anim. Sci. 82: 3380–3387. https://doi.org/10.2527/2004.82113380x
- Ungerfeld R, Gonzalez-Pensado PS (2008). Social rank affects reproductive development in male lambs. Anim. Reprod. Sci. 109: 161–171. https://doi.org/10.1016/j.anireprosci.2007.12.006