Effect of Environmental High Temperature on the Reproductive activity of Awassi Ram Lambs

Hassan Falah Kashef Al-Ghetaa
Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Iraq
Accepted: 20/ 6/ 2012

Summary

The aim of this study was to detect the influence of high temperature during summer and early autumn seasons on the reproductive activity of Awassi ram lambs. Study conducted in Baghdad from June 1st 2011 to Nov. 30th 2011, using thirty Awassi ram lambs aged 11.5±1.5 months. Testis, Seminal vesicle gland and bulbourethral gland dimensions and weights were taken once monthly, cortisol and testosterone hormones were estimated, percentages of dead and abnormal sperms were calculated and histopathological examination was applied on the testes. The results revealed a significant ($P<0.05$) effects of hot season on the genital organ dimensions and weight, and on testicular histological texture leading to sloughing and degenerative changes in seminiferous tubules which causes elevating significantly ($P<0.05$) in percents of abnormal and dead sperms during mid-summer in comparison with fall season. There was decline in testosterone hormone level, while the cortisol hormone levels remains without significant differences. In conclusion: Awassi ram has a great ability to heat stress adaptation to decline the effect of external hyperthermia on reproductive ability as soon as possible, and can resume almost a perfect sexual activity with high fertility rate immediately at the regression of climatic high temperature.
Keywords: Awassi ram lambs, high temperature, testosterone in ram lambs, cortisol in ram lambs, abnormal sperms, testis.

تاثير درجات الحرارة العالية للبيئة في النشاط التكاثري للحملان العواسية

حسن فلاح كاشف الغطاء
فرع الجراحة و التوليد البيطري – كلية الطب البيطري – جامعة بغداد – العراق

الخلاصة

الدراسة الحالية تهدف الى تحديد مدى تاثير درجات المناخ العالية في قابلية تناسل الحملان العواسية في اثناء موسم الصيف و بداية الخريف. اجريت الدراسة في بغداد للفترة من 2011/6/1 الى 2011/11/30 بالاعتماد على ثلاثين من الحملان العواسية بعمر 11.5±1.5 شهر. اخذت قياسات و اوزان الخصية و الغدة الحويصلية المنوية و الغدة البصلية الاحليلية مرة واحدة لكل شهر، وتم حساب تراكيز هرموني الكورتزول و الشحمون الخصوي، فضلا عن دراسة نسب النطف الميتة والمشوهة واجري التقطيع النسجي للخصى لمعرفة مدى تاثير درجات الحرارة العالية في هذه المعايير. اظهرت نتائج الدراسة الحالية ان هناك تاثير احصائي ($P<0.05$) في اوزان وقياسات الاجزاء المختلفة للجهاز التناسلي، وفي التركيب النسجي للخصى اذ ظهرت انسلاخات وتغيرات اضمحلالية في الخلايا الجرثومية المبطنة للنبيبات المنوية الامر الذي ادى الى ارتفاع ملحوظ واحصائي في نسبة الخلايا النطفية الميتة و المشوهة خلال منتصف فصل الصيف عند المقارنة مع فصل الخريف كما وانخفض مستوى الهرمون الذكري في مصل الحملان الا ان مستوى هرمون الكورتزول بقى تركيزه ضمن حدود متقاربة ولم يواجه اي تغيرات معنوية في تركيزه. تستنتج الدراسة ان الحملان العواسية لها القدرة العالية للتاقلم مع درجات الحرارة العالية للمناخ و العودة المبكرة للنشاط التناسلي حال انخفاض درجات الحرارة العالية للجو.
الكلمات المفتاحية: حملان عواسية، حرارة عالية، النشاط التكاثري، الهرمون الذكري للحملان، هرمون الكورتيزول للحملان، تشوهات النطف في الحملان.

Introduction

Natural breeding is the major protocol applied for sheep breeding in most countries. Reproductive performance of rams during mating is very important to have maximum

pregnancy rate in the flock. Testicular development and libido performance of rams or ram lambs are affected by genotype, season, age and ambient temperature (1). In tropical and sub – tropical countries, climatic heat is the major factor restricting animal productivity: growth, milk production and reproduction are impaired because of drastic changes in biological functions caused by heat stress (2). Hyperthermia may be a consequence of environmental conditions, microbial infections and/or hyperthyroidism. Although regulation of body temperature and individual adaptation to environmental climatic changes is well documented, but little known about mechanisms and pathological aspects of hyperthermia (3). Saab *et al*. (4) found that the Awassi ram has a great ability to heat stress adaptation where an exposure to heat stress allows the development of adaptation mechanisms and causes no further effect of subsequent heat stress on spermatogenesis. Scrotal adaptation to heat stress involves the development of sweat glands, mainly following a prior exposure to heat stress, thus allowing for better dissipation of the effects of heat stress on spermatogenesis (5). Heat stress treatment cause decreasing significantly semen concentration and percentage of normal sperms (6 and 7). Hochereau de – Reviers *et al*. (8) found the spermatozoa concentration decreased as a result of a heated environment rather than heated testes, suggesting that stressful environment affect spermatogenesis at a much greater extent than when testes are exposed to localized system and animal thermoregulation. The effect of some continual and crucial environmental factors on the testis characteristics were researched by Koyuncu *et al*. (9), testis diameter generally increased until 34th week of age and showed seasonal variations, the mean testis diameter in inactive rams was smaller than in active rams. Sires and nutrition also effect on the semen characteristics and testis diameter (10 and 11). In Iraq – Baghdad, summer temperature range from 22C^{o} to 29C^{o} minimum to 38C^{o} to 43C^{o} maximum – in the shade temperature higher than 48C^{o} have been reported with June through August usually the hottest months (12).

The aim of this study is detect the influence of high ambient temperature during summer and early autumn seasons on the reproductive activity of Awassi ram lambs through evaluation of normality of sperm morphology supported by hormonal profile estimation to facilitate early male selection using in breeding as well as in artificial insemination.

Materials and Methods

The present study depends on thirty mature local Awassi rams aged 11.5±1.5 months, weighted 33.850±3.090 kg housed in semi – opened shed, in Baghdad (North33^{o}.18$^{=}$, 44^{o}.35$^{=}$ East). All animals involved in this study were fed well with concentrated ration 350 – 400gm/animal/day, in addition to green and dried grasses with water *ad libitum* along a day. The calendar of a study started from 1st June 2011 continues to 30th November 2011. The averages of temperature degree through the study period were obtained from Wunderground. com, which were as illustrated in figure – 1.

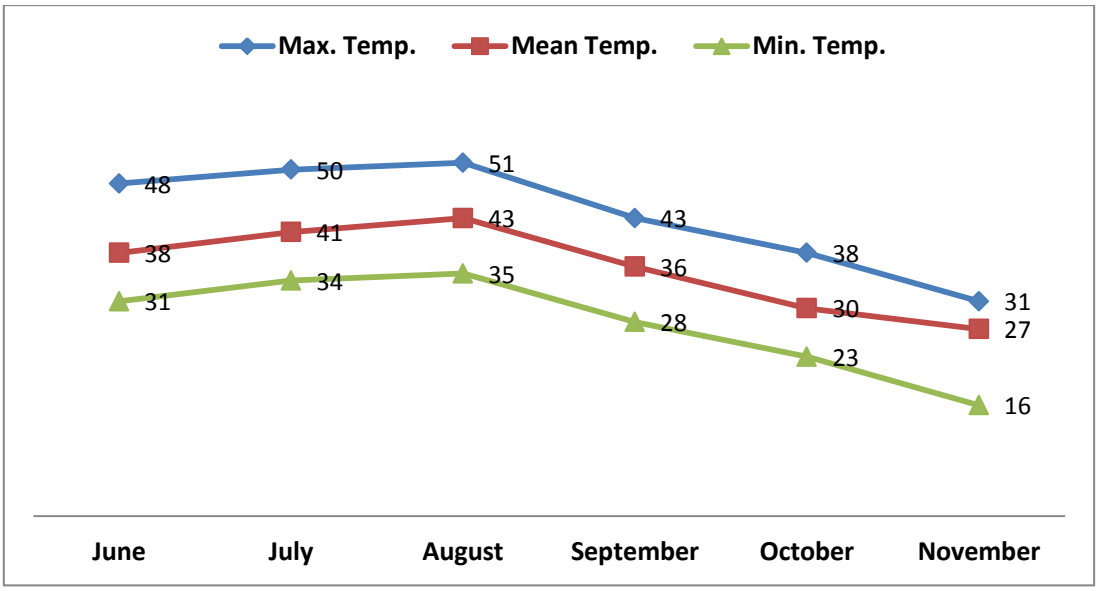

Figure (1): Averages of temperature (C°) along months involved in the study from mid-June/2011 to mid-November/2011 (13).

Measurement of some genital organs dimensions of male reproductive system: Three males were selected randomly to slaughter each month along the study period to obtain the genital organs (testis, seminal vesicle gland and bulbourethral gland) and measure weights and some dimensions such as length and diameter, the parameters were taken from only right sided genital organs by using caliper (14).

Hormonal profile assay: Blood samples were collected twice monthly (every 15 days) along study period from ten males each time to determine the sera concentrations of testosterone and cortisol hormones. Blood sampling were taken via venipuncture and centrifuged at 3000 r/m for 15 minutes, sera were stored at -20C° until hormones assayed with Radio Immuno Assay (RIA) according to (15).

Sperm morphology and live percentages examination: Sperm samples collected by epididymal aspiration by injection of one ml of normal saline into tail of epididymus across scrotum then after 2 – 3 minutes suspended mixture drawn and applied 1 – 2 drops of mixture on glass slide and stained with prepared Eosin1% - Ngrossin5% stain according to Chemineau *et al*. (16) method then left to dry and latterly examine and calculate and identified the abnormalities and live sperms percentage.

Histopathological findings: All separated male reproductive organs were preserved in formalin until preparation of histopathological slides. Right testis of each slaughtered animal (n=3/month) was embedded in paraffin, cut to 3 – 5 micrometer sections, and mounted. After deparaffinization, the tissues were stained with Hematoxylin – Eosin staine (HE) for histological examination according to (17).

Statistical analysis: results were expressed as mean ± Standard error that subjected to statistical analysis using one – way analysis of variance (ANOVA) and LSD, SAS (18) program was used. The significant level considered was ($P<0.05$).

Results and Discussion

Table (1): Effect of heat stress on the testis, seminal vesicle and bulbourethral glands dimensions and weights during study months (M±SE)

	June	July	August	September	October	November
			Testis			
Length (cm)	C 5.802±0.910	BC 6.00±1.007	BC 6.034±1.101	B 6.700±0.076	A 7.666±0.008	A 7.453±0.027
Diameter (cm)	B 3.500±0.500	B 3.589±0.30	B 3.600±0.221	B 3.650±0.046	A 4.075±0.520	A 4.200±0.801
Weight (gm)	C 83.130±1.055	C 82.991±0.86	C 83.44±1.400	B 87.100±0.355	A 91.030±1.06	A 91.100±0.028
			Seminal Vesicle Gland			
Length (cm)	B 3.774±0.008	B 3.702±0.092	B 3.799±0.100	B 3.911±0.022	AB 4.022±0.135	A 4.260±0.030
Diameter (cm)	CD 2.107±0.088	D 1.908±0.530	C 2.395±0.120	B 2.601±0.081	B 2.810±0.100	A 3.326±0.044
Weight (gm)	D 4.098±0.065	E 3.812±0.003	D 4.203±0.111	C 4.953±0.060	B 5.226±0.143	A 5.787±0.169
			Bulbourethral Gland			
Length (cm)	A 1.724±0.009	A 1.720±0.025	A 1.708±0.015	A 1.716±0.011	A 1.753±0.020	A 1.770±0.005
Diameter (cm)	D 1.385±0.120	DE 1.333±0.062	E 1.195±0.105	C 1.592±0.013	B 1.851±0.033	A 2.019±0.089
Weight (gm)	B 3.450±1.008	BC 3.266±0.225	C 3.145±0.002	B 3.519±0.096	A 3.880±0.109	A 3.939±0.082

Different letters refer to significant difference (P<0.05) among study months.

Table – 1 showed there were significant differences (P<0.05) in dimension parameters and weights among testis, seminal vesicle and bulbourethral glands, these differences occurred between a period of breeding and non-breeding seasons with advancing and introducing of rams deeply into breeding season (late summer to end autumn), the highest length (cm) of testis, seminal vesicle and bulbourethral glands were 7.66±0.008 (in October), 4.260±0.030 (in November) and 1.770±.005 (in November) respectively. These results are almost match with finding of Koyuncu *et al*. (9), but not agreed with Elmaz *et al*. (19) which were found positive and significant correlation between testicular measurements and semen characteristics in Kivircik ram lambs, present data were closely to results that recorded by Khalaf and Merhish (20). The significant variation in sizes and weight of genital organs might due to transporting of ram lambs from sexual arrested season into breeding season which accompanied with more androgen (anabolic hormone) secretion which responsible for genital organ development and maturation (21).

Table (2): Effects of heat stress on the cortisol hormone (ng/ml) and testosterone hormone (ng/ml) during study months (M±SE)

	June	July	August	September	October	November
Cortisol hormone (ng/ml)	AB 65.500±0.500	AB 65.800±0.054	A 67.300±0.760	AB 65.600±0.100	B 64.300±0.033	A 66.00±1.000
Testosterone hormone (ng/ml)	D 2.850±0.008	C 4.010±0.560	C 4.300±0.820	B 6.770±0.041	A 8.830±0.200	A 8.510±0.198

Different letters refer to significant difference (P<0.05) among study months.

Cortisol hormone which is known as stressful hormone appeared in highest levels (table – 2) in August and November months, which were significantly (P<0.05) higher than other study months. Generally the cortisol concentration results of this study are higher than that recorded by Roselli *et al*. (22) when estimated the cortisol levels in anesthetized rams. Results reach to fact suggested that sheep especially Awassi have a high resistance to environmental and induced high temperature rather than other species by return the cortisol hormone levels to normal range when the effective conditions had to be chronics status (23; 24 and 25). Gudev *et al*. (26) found the non-specific psychological response in order to reduce the level of calorigenic hormones (cortisol) and to counteract heat-induced inhibition of immune response. The reason of elevated cortisol hormone concentrations in present study could be due to scared animal from restriction and technique of blood sampling which is effect on animal response (25).

Although high ambient temperature during study periods (June – November), the testosterone hormone concentration (table – 2) ascending gradually from lowest levels of out breeding season to reach the maximum concentrations at end of study period (optimal environment to breeding) with statistical differences (P<0.05). Testosterone hormone levels of this study (table – 2), near to that recorded by El-Darawany *et al*. (27) and El-Zelaky *et al*. (28), and exceeded that values recorded by Godkal *et al*. (11) and that recorded by Elmaz *et al*. (19) which estimated it about 2ng/ml in one yearling ram lambs, but were lower incomparison with results recorded by Roselli *et al*. (22) which found testosterone hormone levels during breeding and non-breeding season about 10.5 and 5.0 ng/ml respectively, in male oriented rams. These variations among studies maybe due to different locations among studies involved, because the sheep are seasonal breeder animal get sexual activation during short day period (29).

Androgen in rams mainly testosterone hormone influenced by many factors such as hormonal regulation which once affected by photoperiod (30 and 1). In contrast, Marai *et al*. (31) found that testosterone level was insignificantly affected by season in cross-bred rams. Also testosterone influenced by different types of stressors especially hot-climate, transporting,…….etc. which causes elevating of serum cortisol hormone concentration (21), which in turn effect directly or indirectly on a whole or part of hypothalamus-piuitary-gonadal axis (7; 27; 32 and 33). Indeed, Juniewicz *et al*. (32) and Orr *et al*. (34) found the cortisol hormone interferes with 17α-hydroxylase and C17,20-lyase enzymes activity which responsible for androgen synthesis in leydig cells.

Table (3): Effects of heat stress on the percentages of dead and abnormal sperms during study months (M±SE)

	June	July	August	September	October	November
Dead sperms (%)	**B** **18.150±4.560**	**AB** **35.900±13.097**	**A** **37.019±12.489**	**B** **15.066±2.888**	**C** **7.810±1.980**	C 7.760±1.530
	Morphologically abnormal sperms (%)					
Primary	**B** **7.004±0.070**	**A** **9.031±0.850**	**A** **10.240±2.085**	**C** **4.809±1.023**	**D** **3.750±0.003**	E 1.800±0.003
Secondary	**B** **9.140±1.080**	**A** **12.435±0.610**	**AB** **11.044±2.993**	**C** **5.154±0.983**	**C** **4.553±1.023**	C 4.850±0.989

Different letters refer to significant difference (P<0.05) among study months.

The dead spermatozoa and abnormal morphology sperm (table – 3) were significantly ($P<0.05$) elevated during summer months in comparison with their percentages during fall months. In details, the maximum values of dead and total abnormal sperms were recorded in August versus minimum values of same parameters were in November with significant ($P<0.05$) differences. The major percent of primary abnormal represented by de attached head spermatozoa (free heads) and acrosomes abrasion while, secondary abnormality represented majorly by proximal cytoplasmic droplet and coiled tail sperms. The results of this study (table – 3) exceeding the abnormal spermatozoa percentage recorded by Mohamed and Abdelatif (35) and El-Zelaky *et al*. (28). Seasonal changes in temperature are associated with changes in percentages of abnormal sperms, heat cause detrimental effect even on indigenous stock in hot climatic regions (36 and 4). Also, some other factors might affect on the percentage of normal sperms such as sire (10), body weight (19), age of male (6) and hormonal profile (31; 3 and 23). Caroprese *et al*. (37) suggest, ventilation regimen providing ventilation cycles during the warmest hours of the day and the night may adequately sustain the welfare and production performance of sheep. The explanation of high abnormality in this study may be taken from table – 2 which revealed inadequate testosterone hormone production which has a major role in control of normal spermatogenesis.

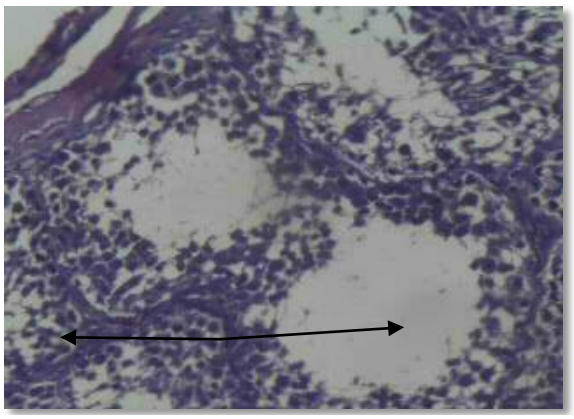

Figure (2): Histopathological section of ram testis (June) showing normal structure appearance of testicular tubules with presence of little sperms (arrows) inside the lumen of tubule. HE stain X200

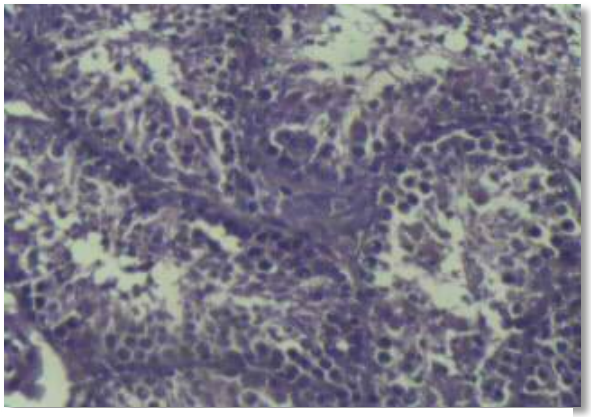

Figure (3): histopathological section of ram testis (July & August) showing degenerative changes and necrosis of tubules with immaturity and no sperms inside the lumen of tubule. HE stain X200

Figure – 2 showed normal structure appearance of testicular tubules of rams obtained in June (transitional period) with presence of little sperms inside the lumen of tubule indicating to exist of a simple cell differentiation of germ cells. Figure – 3 which prepared from testicular tissue taken from ram in August, shows a great damage of epithelial cells (germ cells) and sloughing referring to arrested completely of spermatogenesis process under influence of high-temperature environment (degenerative testicular tissue), this case started from July and get high severity during August.

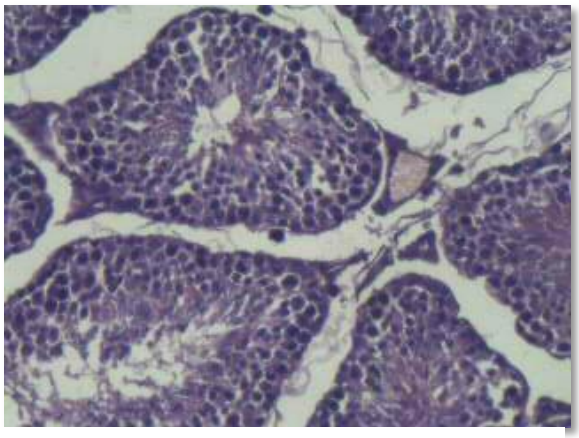

Figure (4): Histopathological section of ram testis (September) showing regenerative changes with maturity but still no numerous numbers of sperms inside the lumen of tubule. HE stain X200

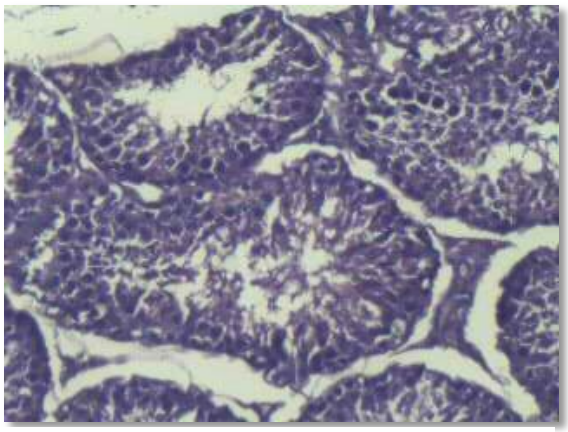

Figure (5): Histopathological section of ram testis (October) showing normal maturation of spermatogonium cells and testicular tubules with presence of numerous numbers of sperms (arrows) inside the lumen of tubule. HE stain X200

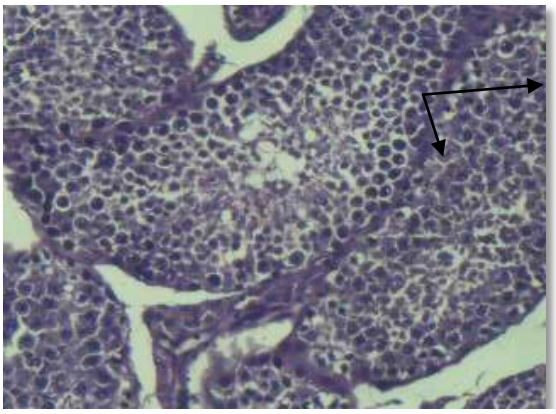

Figure (6): Histopathological section of ram testis (November) showing normal structure appearance of testicular tubules with different stages of spermatogenesis active and large spermatogonium cells (arrows) on a basement of tubule. HE stain X200.

Figures 4 and 5 showed self-cure of testicular tissues of rams from degenerative changes occurred during June and July months (hottest months) and they show regenerative changes accompanied with initiation of normal spermatogenesis process, seminiferous tubules became larger in diameter and composed of many layers of active germ cells and able to excrete spermatozoa. While, at the end of the study (in November) seminiferous tubules structure tend to be perfect in composition, and more active to excrete large quantity of spermatozoa, and germ cells division is more frequent than a previous months (figure – 6), this criteria is agree with increasing of testis dimensions and weight (table – 1) occurred in a same period.

These changes (figure – 3) occurred could be due to loss of gonadotropin hormones (ICSH and SSH: interstitial cell stimulating hormone and spermatogenesis stimulating hormone) support is by increased apoptosis, and as with several other inciting agent, the stage of cell differentiation influences susceptibility (38). Indeed, the melatonin administration has a direct beneficial action on the sperm motility and on other ram sperm characteristics during the non-breeding season, which decreased apoptic-like changes and modulating capacitation and fertilization (3). Heat stress treatment cause significant decreasing in semen concentration, percent of normal sperms, testis weight and number of all germ cells except A0 spermatogonia (7).

In the shadow of all former results, some points should be declare, Awassi ram has a great ability to heat stress adaptation to decline the effect of external hyperthermia on reproductive ability as soon as possible, and can resume almost a perfect sexual activity with high fertility rate immediately at the shallow of high temperature. The dimensions of male genital organs may influenced slightly by season via suppression of steroidogenesis but this does not effect on its texture (except testes). More, the hormonal profile estimation supported with epididymal sperm aspiration and dimension measuring can be uses as a selection criterion to use the ram lambs at an early age. These applications can also be considered to reduce the costs of keeping surplus ram lambs.

References

1. Simitzis, P.; Deligeorgis, S. and Bizelis, J. (2006). Effect of breed and age on sexual behavior of rams. Theriogenology, 65:1480-1491.
2. Marai, I.; Habeeb, A.; Daader, A. and Yousif, H. (1995). Effects of Egyptian sub-tropical conditions and the heat stress alleviation techniques of water spray and diaphoretics on the growth and physiological functions of Friesian calves. J. Arid. Environ., 30:219-225.
3. Sessler, D. (2009). Thermoregulatory defense mechanisms. Critical Care Medicine, 37(7):203-210.
4. Saab, S.; Sleiman, F.; Kallasy, N.; Darweesh, W. and Aad, P. (2011). Effect of adaptation and heat stress on reproductive performances of fat tail Awassi rams in Eastern Mediterranean. Lebanese Sci. J., 12(1):31-44.
5. Setchell, B. (1998). The parks lecture. Heat and the testis. J Reprod Fert., 114:179-194.
6. Alexopoulos, K.; Karagiannidis, A. and Tsakalof, P. (1991). Development of macroscopic and microscopic characteristics of ejaculates from Chios, serres and Karaguniki breed lambs. Theriogenology, 36:667-680.
7. Setchell, B.; Locatelli, A.; Perreau, C.; Pisselet, C.; Fontaine, I.; Kuntz, C.; Saumande, J.; Fontaine, J. and Hochereau-de Reviers, M. (1991). The form and function of the leydig cells in hypophysectomized rams with pituitary extract when spermatogenesis is disrupted by heating the testes. J. Endocrinol., 131:101-112.
8. Hochereau-de Reviers, M.; Locatelli, A.; Perreau, C.; Pisselet, C. and Setchell, B. (1993). Effects of a single breif period of moderate heating of the testes on seminiferous tubules in hypophysectomized rams treated with pituitary extract. J. Reprod. Fert., 97:381-387.
9. Koyuncu, M.; Uzun, S.; Ozis, S. and Duru, S. (2005). Development of testicular dimensions and size, and their relationship to age and body weight in growing Kivirick (Western Thrace) ram lambs. Czech J. Anim. Sci., 50(6):243-248.

10. Colas, G.; lefebre, J. and Guerin, Y. (1990). A study of the transmission from sires to male progeny of seasonal variations in testis diameter and percentage of abnormal spermatozoa in Ile-de-France ram. Reproduction, Nutrition and Development, 30: 589-603.
11. Godkal, O.; Atay, O.; kum, S.; Yilmaz, A. and Eren, V. (2012). The effect of supplemental vitamin E on reproductive development of pre pubertal Karya male lambs. Egyptian J. Sheep and Goat Sci., 7(1): 11-17.
12. Encyclopedia Nations (2012). http://www. nationsencyclopedia. com / geography / indonesia - to-mongolia/iraq.html.
13. Wundergroud.com/history/airport/KQTZ/2011/6/2/monthlyhistory/html.
14. Salhab, S.; Zarkawi, M.; Wardeh, M; Al-Masri, M. and Kassem, R. (2001). Development of testicular dimensions and size, and their relationship to age, body weight and parental size in growing Awassi ram lambs. Small Rum. Res., 40:187-191.
15. Berga, S. and Dainiels, T. (1991). Use of the laboratory in disorder of reproductive neuro-endocrinology. J. Clin. Immuno. assay, 14:23-38.
16. Chemineau, P.; Cogin, Y.; Guerin, Y.; Orgeure, P. and Valet, J. (1991). Training manual on artificial insemination in sheep and goats. FAO Animal Production and Health Paper, No.83.
17. Luna, L. (1968). Manual of histological staining methods of the armed forces. Institute of pathology, Newyork, McGraw-Hill Book Company, Pp:222-223.
18. SAS. (2010). SAS/STAT User's guide for personal computers. SAS Institute Inc. Cary, NC. USA.
19. Elmaz, O.; Cirit, U. and Demir, H. (2007). Relationship of testicular development with age, body weight, semen characteristics and testosterone in Kivircik ram lambs. South Africa J. Anim. Sci., 37(4):269-274.
20. Khalaf, A. and Merhish, S. (2010). Anatomical study of the accessory genital glands in male sheep *(Ovis aries)* and goats *(Caprus hicus)*. The Iraqi J. Vet. Med., 34(2): 1-8.
21. Pineda, M. (2003). Male reproductive system. In: McDonald's Veterinary Endocrinology and Reproduction. Pineda, M. and Dooly, M. (Eds.), 5th Ed. Iowa State Press, USA, P: 239.
22. Rosella, C.; Stormshak, F.; Stellflug, J. and Resko, J. (2002). Relationship of serum testosterone concentrations to mate preferences in rams. Biol. Reprod., 67:263-268.
23. Habeeb, A.; Marai, I. and Kamal, I. (1992). Heat stress. In: Farm Animals and the Environment, Phillips, C. and Piggins, D. (Eds.), CAB International, Walling Ford, UK., Pp: 27 – 47.
24. Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. Livestock Prod Sci., 67: 1-8.
25. Broom, D. and Kirkden, R. (2004). Welfare, stress, behavior and pathophysiology. In: Veterinary Pathophysiology, Dunlop, R. and Malbert, C. (Eds.), 1st Ed. Blackwell Publishing Ltd. Iowa, USA, P:337.
26. Gudev, D.; Popova-Raltcheva, S.; moneva, P. and Johnson, H. (2005). Endocrine changes in two behavioral types of cows exposed to heat. Biotech Anim Husb., 21(5-6):54-51.
27. El-Darawany, A. (1999). Improving semen quality of heat stressed rams in Egypt. Indian J Anim Sci., 69:1020-1023.

28. El-Zelaky, O.; Khalifa, E.; Mohamed, A.; Mohamed, K. and Hussein, A. (2011). Productive and reproductive performance of Rahmani male lambs fed rations containing Jatropha cake. Egyptian J Sheep and Goat Sci, 6(2):15-24.
29. Bearden, H. and Fuquay, J. (1992). Spermatogenesis and maturation of spermatozoa. In: Applied Animal Reproduction. Bearden, H. and Fuquay, J.(Eds.), 3rd edition, Printice Hall Englewood Cliffs, NewJersy. USA. Pp:67.
30. Lincoln, G.; Townsend, J. and Jabbour, H. (2001). Prolactin actions in sheep testis: A test of priming hypothesis. Biol.Reprod., 65:936-943.
31. Marai, I.; El-Darawany, A.; Fadiel, A. and Abdel-Hafez, M. (2008). Reproductive performance traits as affected by heat stress and its adult alleviation in sheep. Tropical and Subtropical Agroecosystems, 8:209-234.
32. Juniewicz, P.; Johnson, B. and Bolt, D. (1987). Effect of adrenal steroids on testosterone and luteinizing hormone secretion in the ram. J Androl., 8:190-196.
33. Elmaz, O.; Dikmen, S.; Cirit, U. and Demir, H. (2008). Prediction of post pubertal reproductive potential according to pre pubertal body weight, testicular size and testosterone concentration using multiple regression analysis in Kivircik ram lamb. Turkey J Vet Anim Science., 32(5):335-343.
34. Orr, T.; Taylor, M.; Battacharyya, A.; Collins, D. and Mann, D. (1994). Acute immobilization stress disrupts testicular steroidogenesis in adult male rats by inhibiting the activities of 17α-hydroxylase and 17,20-lyase without affecting the binding of LH/hCG receptors. J Androl., 15:302-308.
35. Mohamed, S. and Abdelatif, A. (2010). Effect of level of feeding and season on thermoregulation and semen characteristics in Desert rams *(Ovis aries)*. Global Veterinaria, 4(3):207-215.
36. Akpokodje, J.; Dede, T. and Odili, P. (1995). Seasonal variations in seminal characteristics of West Africa dwarf sheep in the humid tropics. Tropics Veterinarian, 3:61-65.
37. Caroprese, M.; Abenzio, M.; Marino, R.; Mscio, A.; Santillo, A. and Sevi, A. (2008). Strategies to reduce heat stress in sheep housing. In: international Conference, Ragusa-Italy, 15 – 17th September 2008.
38. Troedsson, m and Madill, S. (2004). Pathophysiology of the reproductive system. In: Veterinary Pathophysiology, Dunlop, R. and Malbert, C. (Eds.), 1st edition, Blackwell Publishing Ltd. Iowa, USA, Pp:236-240.