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The effect of different seasons on the seminiferous tubules and leydig cells of the testis for the adult rabbit of the local breed (Histological study)

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Abstract
The season is an important factor determine the efficacy of the sexual activity of the adult males of different animal species, in this study, Sixteen adult male rabbit of local breed were used to investigate the effect of the different seasons of the year on the histology of the testicular tissue, particularly the leydig cells. Four animals were killed in each season and segments of testes processed by using histological technique. The results were indicated that the seasons winter and summer affect the seminiferous tubules reflected by subpopulation of different germ cells such as spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Leydig cells were atrophied with deeply basophilic nuclei associated with poorly blood capillaries in between, otherwise the seasons spring and autumn effect on the same germ cells and leydig cells, but in this case there was increase in the population of germ cells in the seminiferous tubules and the leydig cells were hypertrophied with the presence of cytoplasmic fat droplets and its nuclei were euochromatic, The blood capillaries in between these cells were abundant, these events indicated the increase activity of testicular tissue in both seasons, the (spring and autumn), While in winter and summer, this phenomena was contra versa. The present study was indicated that the spring and autumn seasons are the best for the progressed activity of the leydig cells and germ cells of the testicular tissue of the adult rabbit than the winter and summer seasons.

Introduction
The investigation of the seasonal alteration of the leydig cells were studied in present study (1), their study were associated with the histomorphological changes of leydig cells in Japanese jungle crows. (2) in the equatioral birds, (3) in the male ostrich and (4) in the Japanese quills. The present study was designed to investigate the seasonal alterations on the leydig cells of the local breed adult rabbit.

Literature Review
Dittami (5) showed avian seasonal alteration of testosterone and leutenizing hormone (LH) levels at the cycle of the reproduction and these data were supported by (6) in the japanese common pheasants .
Expression of estrogen help the growth and differentiation for interstitial cells in between seminiferous tubules of testis at the annual reproductive cycle in the Atlantic cod (78) suggested that testosterone in concerned with spermatogenesis associated with general histomorphological changes of leydig cells like presence of the lipoidal contents, cholesterol - positive prior to onset of preproductive season.
Leydig cells give rise for new generation of immature interstitial cells at the end breeding season or peroid (9). Lake (10 stated that when the sexual season was approached, the spermatogenic activity in the seminiferous tubules are building up and thse are lipids and cholesterol.as main components of it.
Muhammad and Masato (1) attributed a new generation of leydig cells are derived from fibroblast of the interstitial CT in between seminiferous tubules of the Japanese crow. (11) evaluated the effect of season on the testicular leydig cells in the Egyptian nubian goat, they found that the thickness of seminiferous tubules was maximal during autumn and summer .
Nagwa and Ahmad (12) demonstrated the effect of short photoperiod exposure on the leydig cells in rat testis with decrease of the testosterone secretion, they found hypo function of the leydig cells with drop of reproduction and regression
Leydig cells showed cytoplasmic hypertrophy during the breeding period (summer and autumn) and lipid inclusion, dominant organelles and the nuclei became heterochromatic and irregular (13).

Materials and Methods
Sixteen adult male local breed rabbit were used in the present study, housed freely since august 2014 until August 2015, they were subdivided into four groups (4) for each season of study, four males were killed in each season, the scrotum was opened through an incision on the ventral surface. After that, the testes were immediately removed and washed in running water to exclude the debris and blood clot. Each testis was sectioned into a segments of .05cm thickness, placed in Bouins solution for fixation 24 hours, dehydrated in a graded alcohol series from 50%, 70%, 80%, 90% and 100% 4 hours for each step, then cleared in xylene two changes 2hours for each step, embedded in paraffin and cut at 6 micrometer thickness using manual microtome (14). The sections were stained by Haematoxylin and Eosin (H&E) and finally examined by light microscope at different magnifications .

Results and Discussion
The gross anatomy of the rabbit testis revealed that it was oval in shape, the whitish color of it was due to the presence of tunica albuginea which covers the whole testis and there was a network of blood vessels through this layer. The testis was attached to the head of the epididymus, body and the tail which continued with the vas deference in the spermatic cord (Fig.1). These results was not away from the description of many scientists about the gross anatomy of the testis in the mammals such as (15).
The group of animals which were examined at winter

revealed that the seminiferous tubules were less dialated and the different types and stages of germ cells were demonstrated, the leydig cells were atrophied and appeared as small mass in between the seminiferous tubules and rarely seen the capillaries between and around these cells, the nuclei of these cells were heterochromatic and the chromatin was highly basophilic.(Fig.2), the testicular tissue and the leydig cells mainly concerned with the seasonal changes (11). Suchcela et al (16) referred that the leydig cells are affected when induced by exposure to the chronic fluoride toxicity and the leydig cells become shrunken, this result in winter confirm the study of (12), when studied the leydig cells in the testis by ultrastrucure and immunohistochemistry, they found that during winter , there was detection in subpopulation of the leydig cells per tubular cells.

The group which were examined in spring revealed that seminiferous tubules were more dilated to that of group winter , the different stages of spermatogenesis and spermiogenesis were detected , also the presence of spermatids near the center of the lumen and spermatozoa in the center of each seminiferous tubule were present, the interstitial connective tissue was enriched with leydig cells of large size associated with the presence of cytoplasmic droplets of fat looked like spaces and the nuclei of leydig cells were euochromatic and the chromatin in side these nuclei appeared pale color , the capillaries also present in between the cells and around it (Fig.3). The presence of the cytoplasmic droplets in the leydig cells is an indication for the increase activity of the smooth endoplasmic reticulum of these cells which are responsible for the production of the testosterone , this result confirm the result of (17 and 18) when referred that cytoplasmic hypertrophy of leydig cells were demonstrated during summer and autumn, but in the present study, found these cytoplasmic droplets and hypertrophy of the cells in the spring season ..

The group that examined in summer referred that the seminiferous tubules were dilated, but the population of the spermatogonia, primary and secondary spermatocytes were scanty which were associated with decrease number of the spermatids and spermatozoa. The leydig cells in between the tubules were atrophied with dense basophilic nuclei and its groups were few with poor blood capillaries, also the interstitial connective tissue appeared scattered and few. (Fig.4.), involving the testicular tissue, the present study not confirmed the result of (17) when they referred that cytoplasmic hypertrophy of leydig cells were demonstrated in the autumn and summer , but in agreement with the present study for the season autumn., the group of autumn demonstrated that the seminiferous tubules were moderately dilated and the different germ cells inside the seminiferous tubules were clearly seen with presence of a mass of spermatozoa inside the lumen of these tubules. The leydig cells were present in between the seminiferous tubules as a masses of three to four cells in each group associated with the presence of blood capillaries a round and in between it. The nuclei of the leydig cells were pale in stain with the presence of small cytoplasmic droplets in the form of white vacuoles and the interstitial connective tissue was packed with germ cells of different stages that included fat droplets, foamy appearance. (Fig.5), involving the testicular tissue, the present study confirmed the results of certain investigators about the sesonal effects on the leydig cells in the Japanese quails(4). Loft (2) in the equatorial birds. (5) in the geese, (6) in the Japanese pheasant and Nagasawa et al (7), all those investigators referred that the leydig cells are affected by season and this affection was related with the ultrastructure of the smooth endoplasmic reticulum activity associated with the serum testosterone and lutenizing hormones levels at the cycle of reproduction. The present study concluded that histological changes of the testicular tissue of the rabbit was clearly affected with the season and this phenomena was demonstrated via alteration of the population of the germ cells in the seminiferous tubules and the leydig cells by its changes in the size and presence of the cytoplasmic fat droplets which reflect the activity of the cells in definite seasons in the year.

Finally, the presence of euochromatic nuclei in the leydig cells of the rabbit testis at seasons spring and autumn was indicating for the more activity of these cells in the secretion of the androgens, and this concept in corresponding to that data reported by (19) when also said that heterochromatic nuclei are indicating for inactive cells ,as well as the presence of fat droplets intracytoplasmic leydig cells is a signal for increase activity of the smooth endoplasmic reticulum for the secretion of that hormone (12).

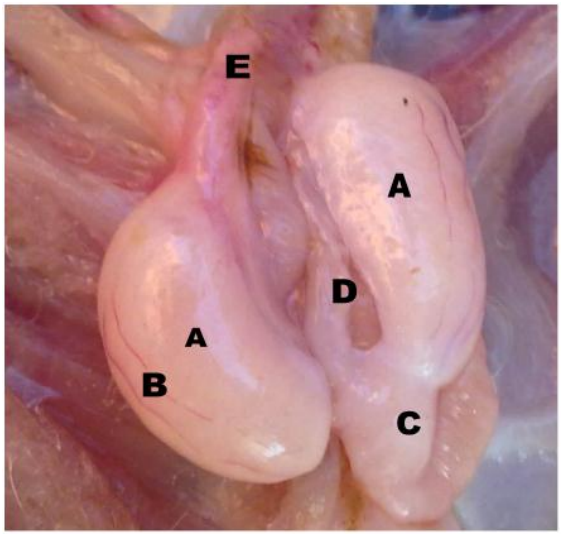

Figure (1): Testis of rabbit. (A) tunica albuginea. (B). Blood vessels. (C).Tail of epididymis (D) Body of epididymis (E) Spermatic cord.

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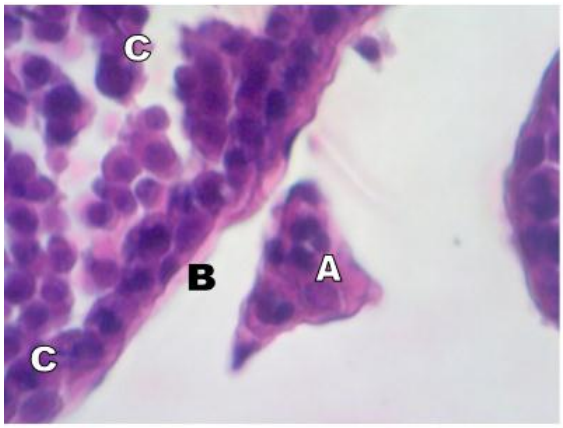
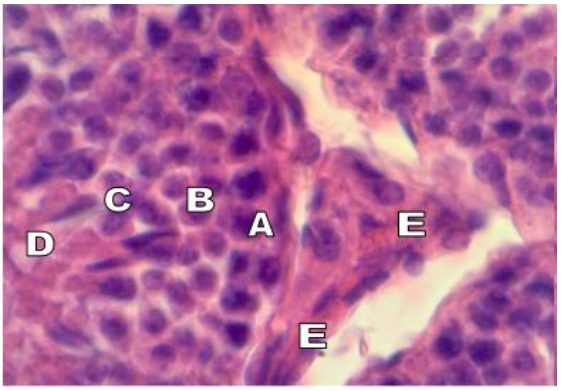


Figure(2)Winter group of rabbit testis .(A) leydig cells with heterochromatic nuclei, (B), thickening basement membrane (C)few germ cells in the SNT.(H&E stain, 40X).



Figure(3).Spring group (A)Spermatogonia, (B)Primary spermatocytes (B) Secondary spermatocytes (D)Spermatids (E) Hypertrophied Leydig cells with euochromatic nuclei (H&E stain 40X).

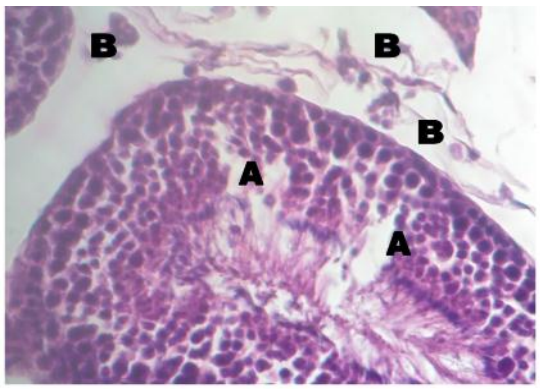


Figure (4) Summer group showing subpopulation and loosening of the germ cells from each other (A)and the leydig cells appeared scattered and atrophied (B) with density of nuclei in the interstitial CT.(H&E stain, 20X).

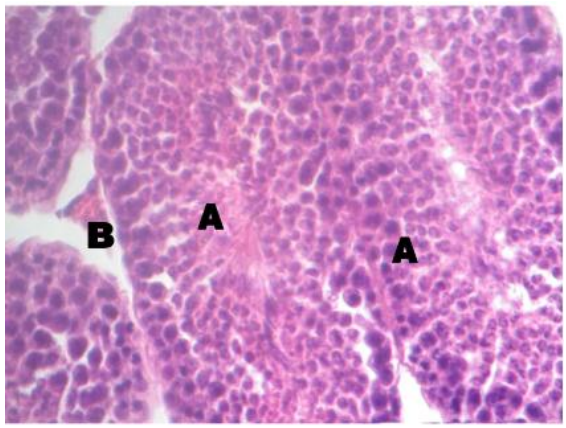


Figure (5).Autumn group indicating the density of germ cells in the SNT(A) and the leydig cells (B) with euochromatinic nuclei of large size.(H&E stain ,20X)

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تاثير المواسم المختلفة على النبيبات الملفوفة المنوية وخلايا ليدك في خصى الارانب المحلية (دراسة نسيجية)

ادريس خلف ثامر

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الملخص

الموسم عامل مهم في تحديد فعالية النشاط الجنسي للذكور لمختلف اصناف الحيوانات, ستة عشر ارنب ذكر بالغ من سلالة محلية تم استخدامها لبيان تأثير الفصول المختلفة في السنة على التركيب النسيجي للخصية خاصة خلايا ليدك.

في كل فصل تم قتل اربع حيوانات وتم اخذ قطع من الخصى وتمريرها بأجراء التقنية النسيجية عليها.

بينت النتائج بأن موسمي الشتاء والصيف كانا اقل تأثيرا على النبيبات المنوية الملفوفة وانعكس ذلك في قلة اعداد الخلايا الجرثومية فيها مثل سليفات النطف, الخلايا النطفية الابتدائية والثانوية, الطلائع النطفية والنطف, كما ان خلايا ليدك كانت ضامرة الحجم وفيها انوية داكنة الصبغة مع وجود قلة في اعداد الشعيرات الدموية حولها, من ناحية اخرى فأن موسمي الربيع والخريف اظهرا تأثير على الخلايا الجرثومية المذكورة في النبيبات وخلايا ليدك بينها حيث تبين زيادة في اعداد تلك الخلايا مع وجود تضخم في خلايا ليدك ووجود قطيرات دهنية في السايتوبلازم وانوية خلايا ليدك كانت باهتة الصبغين كما ان هناك وفرة في الشعيرات الدموية بين خلايا ليدك.

تلك الاحداث بينت نشاط نسيج الخصية الارانب في موسمي الربيع والخريف بينما موسمي الشتاء والصيف فأن تلك الظاهرة كانت معكوسة.