Iraqi Journal of Veterinary Medicine Vol. 34, No. 1, 2010

Prenatal Study of Testes Growth and Histological Development

1- Fetal Sheep.

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Accepted date 4/4/2010

Summary

This study was conducted on 200 samples of females Karadi sheep genitalia contains 207 fetuses . These samples were collected from slaughter house in Sulaimani reigion during the period from June 2007 to the end of May 2008. Sheep genitalia consisted of 193(96.5%) single and 7(3.5%) twin fetuses, amonge them 122 (58.92%)and 84(41.06) were in the right and left horns ,respectively. The genitalia that contain fetuses with clear external sex organs was 141, of these 74 male and 67 female fetuses different Crown Rump Length (CRL), while 66 fetus their sex could diagnosis after abdominal opened. The results showed the weight of the fetal body and testes as well as the length of scrotum were increased with an increasing of the fetal CRL. The appearance of the fetal testes in the inguinal ring was when CRL=22cm .80 day; in the scrotum 32 cm. 100 day ; Histological sections of the fetal testes were showed the density of fibroblast layer and connective tissue capsules as well as the appearance of seminiferous tubules containing a few spermatogonia cells and small sertoli and leydig cells at early CRL=10 cm 55 day; , with increasing fetal CRL the seminiferous tubules filled with different types of spermatogonia and spermatocytes cells as well as increasing in the number of sertoli and leydig cells were recorded at CRL= 20.3 cm. 75 days; While the metamorphosis spermatid were seen at CRL=24cm . 80-85 day; Conclusions : Fetal sheep seminiferous tubules early in gestation contain different types of spermatognium and spermatocyte cells appear in the testes after 50 days, and metamorphosis spermatid were seen after 80 days of intra uterine life no sperm was seen in fetuse testes.. It was concluded that the fetal testes in early gestation could be secreted testosterone which lead to increases in fetal testes development and growth of the germinal and somatic cells.

دراسة النمو والتطور النسيجي لخصى الاجنة 1- اجنة الاغنام

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الخلاصه

تمت دراسة 200 عينة من ارحام الاغنام الكرادي التي تحوي على 207 جنين منها 7 ارحام تحوي على توائم (3.5 %) . اعداد الاجنة في القرن الايمن 122 (58.93 %) والقرن لايسر 85 (41.06 %) . اعداد الاجنة التي تم تشخيص الجنس فيها عيانياً ظهور الاعضاء التناسلية كانت 141 جنين منها 74 من الذكور هذه الاجنة جمعت من مجزرة السليمانية وذات طول تاجي عصعصي مختلف . بينت نتائج التجربة بان اوزان الاجنة وا لخصى واطوال كيس الصفن تزداد

بزيادة طول التاجي العصعصي للجنين / سمز وجدت خصى الاجنة بطول 22 سم , 80 يوم في الحلقة الاربية وداخل كيس الصفن للاجنة بطول 32 سم في 100 يوم . الدراسة النسجية لخصى الاجنة عندما يكون طول التاجي العصعصي 6 سم تقريباً تحوي على نبيبات منوية فيها بعض الخلايا الجرثومية وخلايا ليدك خارج النبيب .وبزيادة طول التاجي العصعصي تحصل الزيادة في انواع الخلايا الجرثومية داخل النبيب وخلايا سيرتولي , وتزداد اعداد و احجام خلايا ليدك . ظهور طلا ئع النطف عند طول 24 سم بعمر 80 – 85 يوم تقريباً . نستنتج من هذه الدراسة بان خصى الاجنة لها القابلية على افراز هرمون الشحمون الخصوي الذي يودي الى زيادة في حجم الخصى بزيادة نسجية في الخلايا الجرثومية والجسمية المختلفة في خصى الاجنة وظهور طلائع النطف في الثلث الاخير من عمر الجنين . ويساعد هرمون الشحمون الخصوي في نزول الخصى الى كيس الصفن.

Introduction

Karadi sheep are fertile small ruminants breed in Sulaimani ق egion; they are used mainly for the production (1) . In the adult male of many mammalian species, libido and testicular function, which are thought to be mediated through alterations in gonadotrophin secretion from the pituitary gland. The development and function of the testes in sheep fetuses are known to be critically depends on circulating fetal gonadotrophin concentration (2). McNatty(3) reported that the physiologically relevant stages of fetal development are includes: after sexual differentiation, early central nervous system (CNS) androgen-responsive phase and day 50 of gestation. Middle of the CNS androgen-responsive phase, before pituitary function (4 and 5 ; day 65 of gestation); and after the onset of gonadotrophin secretion (5;day 110 of gestation.). the fetal plasma testosterone concentrations increases from 0.6 nmol/L at day 50 of gestation to 1.5 nmol/L day 65 to 3.7 nmol/L day 110 of gestation (6 and 7). There was no data available considering the size, location, of fetal testes as well as the histological study of the development of the fetal testes. The present study was designed to investigate the histological development of fetal testes and to determine the location, size, and migration of fetal testes throughout the majority of intra uterine life of Karadi sheep. Materials and Methods

Two hundred(200) samples of sheep genitalia contains one or two foetuses. were collected from a new slaughter house in Sulaimani Governorate during the period from June 2007 to May 2008. . The breed of the slaughtered sheep were mostly Karadi (1). Immediately after evisceration, the genitalia were separately placed in plastic bags and transported to a laboratory of Veterinary Teaching Hospital at the College of Veterinary Medicine . The genitalia containing foetuses were freed carefully from extraneous tissues .

The foetuses Crown Rump Length (CRL) was measured using Varner calibre (Guogence Battery SR441.55V). Age of foetuses (stages of gestation) were estimated by measuring foetal CRL, and the age of the foetuses in days was calculated, according to Noakes et al. (8), Foetal sex was determined by external examination of the foetal external genitalia in the 74 males and 66 females appeared macroscopically clearly. The weight and location of the foetous were recorded , the 66 sheep foetuses which their sex could not be determined externally ,the abdominal cavity was opened to determine the location of the testes. The testis descending from the kidney was recorded . Both testes were weighed by an electric sensitive balance ,Then the testes were fixed in 10% neutral buffered formalin for histological examination according to Luna (9). The blocks were cut by rotary microtome (Mod.1130/Biocut) into sections (5-6) micron

The SAS(10) program was used to find out the effect of group and age of different traits. Least

significant difference (LSD)was used to compare the significant differences between the different meanes.

RESULTS

The total number of sheep genitalia that contains 207 fetuses was 200,of them 7 contain twin fetus. The total number of genitalia contained 141 fetuses with clear external sex organs was 140 (one genitalia was contain 2 fetus). The total number of male fetuses of different CRL recorded was 74(35.74%)and the number of genitalia that contained 66(32.03%) fetuses whose external fetal sex organs could not be detected (under 6 cm CRL) was 60 genitalia(6 genitalia was contain 2 fetus)

Table 1. shows that the fetal weight was steadily significantly increased from 2.5cm to 43.86cm CRL. The fetal external sex organs in male could be determined from 7.32cm CRL.

Table 1: The relation of ovine fetus weight (gm), fetal sex and fetal location in various fetal ages(in days) in single and twin Pregnancy(± S.E.)

No. of fetuses	Fetal CRL	Fetal Age/ day	Fetal weight gm	Fetal Sex		Fetal location Horns	
				M	F	R.	L.
12 Single	1.7	39.27	N 0.76±0.042	-----	-----	7	5
12 2 Twin,8 Single	2.5	41	N 2.01±0.074	-----	-----	7	5
12 Singles	3	42	N 3.06±0.06	-----	-----	8	4
10 3 Twin,4 Single	3.81	43.73	N 4.44±0.39	-----	-----	6	4
10 1Twin,8 Single	5.35	46.89	N 9.05±0.13	-----	-----	8	2
10 Single	6.32	48.8	N 10.35±0.08	1	-----	6	4
8 Single	7.32	51.1	MN 21.49±0.19	5	3	5	3
8 Single	8.5	53.5	LMN 34.8±0.25	4	4	4	4

13 1Twin, 11Single	10.8	56.9	KLM 51.22±0.21	6	7	8	5
10 Single	11.3	59.4	KL 61.74±0.28	6	4	7	3
10 Single	12.3	61.5	JK 74.75±0.23	4	6	7	3
9 Single	13.3	63.5	IJ 108.25±0.38	6	3	6	3
10 Single	14.3	65.7	I 120.6±0.28	4	6	5	5
9 Single	15.2	67.6	I 134.6±0.30	5	4	5	4
10 Single	17.4	72.1	H 170.8±0.51	4	6	6	4
9 Single	19.2	76.1	G 237.27±0.58	6	3	5	4
9 Single	21.7 20-23	81.2	F 295.55±0.98	5	4	4	5
9 Single	24.44 24-25	86.94	E 444.33±0.98	4	5	6	3
7 Single	27.64 26-29	93.8	D 795.86±1.87	3	4	3	4
6 Single	32 30-34	104 98.7-107	C 1125.71±1.29	3	3	3	3
6 Single	36.5 35-38	112.4	B 1691.67±4.29	3	3	3	3
7 Single	43.86 43-45	127.8	A 2672.86±2.41	5	2	3	4
LSD			*37.989				
Total				♂ 73	♀ 67	122	84

Table 2 Demonstrates that the weights of fetal testes and length of fetal scrotum was steadily increased from 6 cm CRL to 45cm CRL, it also shows the location of fetal testes and the descending of the testes from the kidney in different CRL (different ages), when CRL is 7.32cm .48 day; the location of the testes is close to the kidney, whereas in 13cm CRL 63 day ; the location of the testes is 0.90cm away from the kidney with clear external penis, but in 24cm

CRL.86 day ; the testes are located in the scrotum and/or inguinal ring.

Table 2.Showed the Fetal testes weight, its descending and location during intra uterine life.

Fetal NO.	CRL (cm)	Testes weight (gm)	Scrotum Length (cm)	Testes location
5	7.32	0.015	0.3	Close to Kidney
3	10	0.035	0.35	Close to KIdney
5	11	0.046	0.5	0.2 cm far from Kidney
3	12	0.056	0.6	0.5 cm Far from Kidney
4	13	0.06	0.75	0.9 cm Far from Kidney with Clear Penis
4	15	0.064	1.2	1.2 cm Far from Kidney
4	16	0.068	1.7	1.2 cm Far from Kidney
5	18	0.073	1.6	1.5 cm Far from Kidney
3	19	0.093	1.58	Near Inguinal Ring
4	20	0.19	1.6	Near Inguinal Ring
4	22	0.205	2.1	Inguinal Ring
4	24	0.23	2.27	In scrotum or Inguinal Ring
4	27	0.423	2.57	In Scrotum or Inguinal Ring
4	29	0.72	2.62	In scrotum or inguinal Ring
2	32	0.85	2.81	In Scrotum
2	34	1	3	In Scrotum
3	37	1.04	3.1	In Scrotum
3	40	1.277	3.5	In Scrotum
4	45	1.87	3.75	In Scrotum

***** Testes weight included epididymis.

The histological development of fetal testes were followed up from early crown rump length (CRL) about 8.5-10cm, to 43cm(56- 126 days).At early stages, CRL= 10cm, the sections showed the testes with few seminiferous tubules, containing few spermatogonium cells, immature sertoli and leydig cells (Fig.1). The seminiferous tubules appeared embedded in loose connective tissues and stroma which also contained vessels, and small leydig cells. When fetal CRL=12 cm , the section also,shows an outer thin layer of fibrous capsule, tunica albuginea, are surrounded the testis, and divided the interior of the testis into lobuli . The testis also showed convoluted seminiferous tubules with spermatogonia cells. Crose sections were showed the density of fibroblast layer and connective tissue capsules, they are usually concentrated on the posterior aspect of the testis adjacent to the epididymis. The presence of Vas deferens with thick layers and the quite small lumen, it passes parallel to the testis (Fig.2; CRL=14cm). With an increased fetal CRL=19cm, the section appeared with seminiferous tubules filled with different types of spermatogonia cells, tubules shows spermatocytes cells ,with an increases in the number of sertoli cells. Leydig cells as arranged in cord between tubules, and tubules are covered externally by fibrous tissues and fibroblast cells (Fig.3). When the fetus is about 29cm CRL ,85 day; histological sections shows an increases in component cells of spermatogonium, spermatocytes, sertoli cells and metamorphosis spermatid inside tubules. The presence of crowded leydig cells

between tubules can be noted (Fig.4). The seminiferous tubules lined by complex germinal epithelium. The different types of metamorphosis spermatid developed in seminiferous tubules CRL=34cm.100-110 day;(fig.5).

The epithelium rests upon a thin basal lamina and is covered externally by fibroblast and cells with the chara al sheep is more than 29-34cm. A netv r connective tissue 43cm CRL.,120 day. (fig.6).

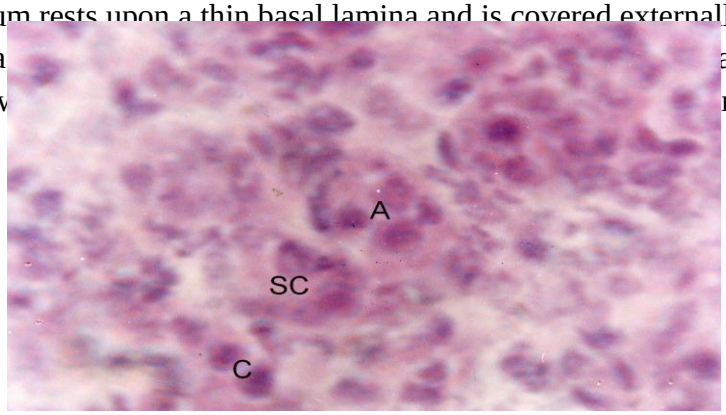

Fig. (1).Fetal Sheep Tests CRL= 10 cm. H and E. X 100 A-Seminiferoustubules C-Leydig cells SC –Sertoli cells

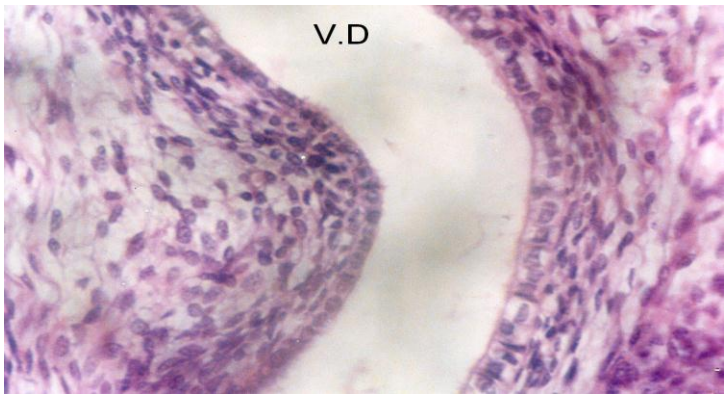

Fig. (2). Fetal Sheep Testes CRL= 14cm. H and E. x 100.
VD = Vas deferens.

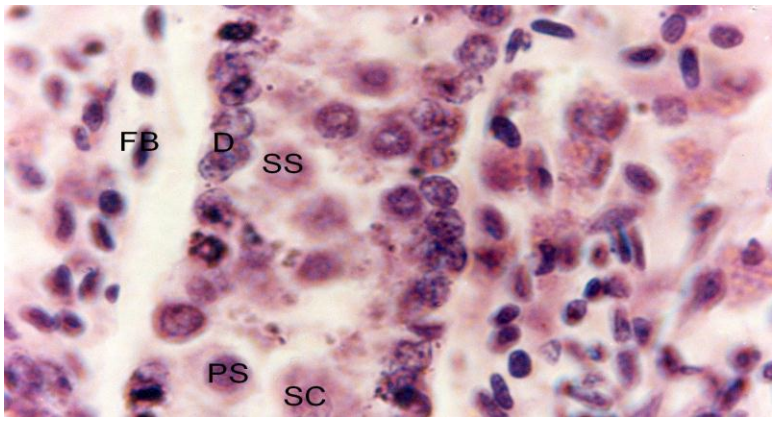

Fig. (3) . Fetal Sheep Testes CRL = 19cm . H and E. X 100.
D= Spermatogonium cells SS= Secondary spermatocyte
SC= Mature Sertoli Cell PS= Primary spermatocyte.
FB= Fibroblast

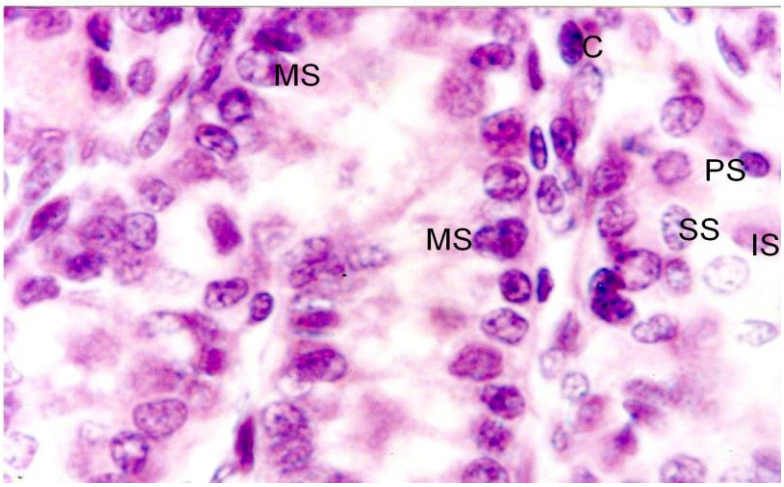

Fig. (4). Fetal Sheep Testes CRL= 29 cm. Histological sections .H. and E. X 100 .
C= Laydig Cell . PS= Primary Spermatocyte . MS=Metamorphosis Spermatid
IS= I ntermediate Spermatogonium SS= Secondary Spermatocytes

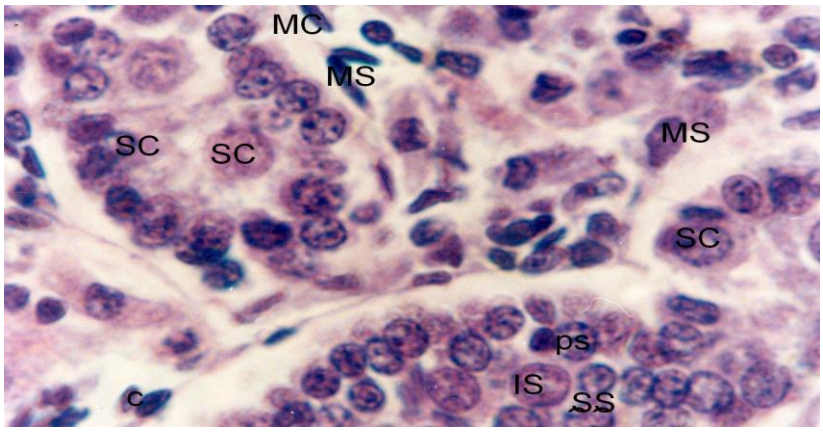

Fig.(5).Fetal sheep CRL= 34cm. H. and E. X100.
MC=Myoid Cell. MS= Metamorphosis Spermatide. SC = Sertoli Cell. PS = Primary spermatocyte. IS= Intermediate spermatogonium . SS= secondary spermatocyte.

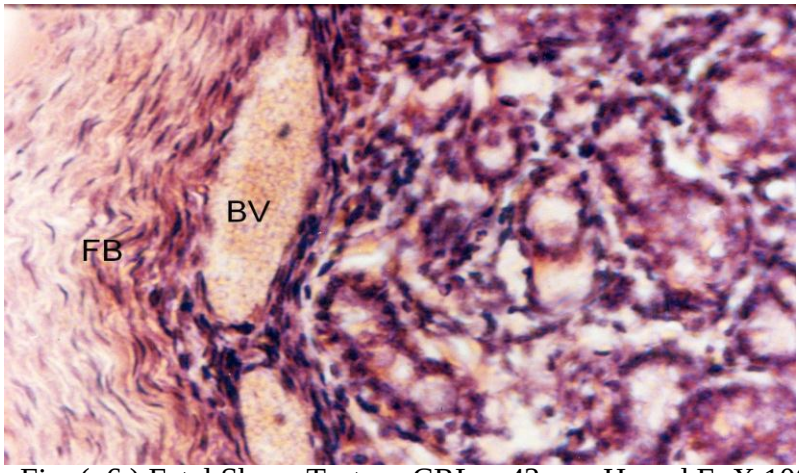

Fig. (6) Fetal Sheep Testes.. CRL = 43 cm. H. and E. X 100. Fig.(6). Tunica Albugainea FB= Fibroblast.
Tunica Vasculosa = B.V.

DISCUSSION

This is the first study that follow up fetal testis weight, migration and histological development of different germinal cells in the seminiferous tubules and Leydig (interstitial) cells during intra uterine life. There is a few scattered reports about the histology of the fetal sheep testes in pregnant ewes.The results of tables 1 and 2 explained the fetal CRL measurements were directly correlated with fetal weigh ,testes weight and the length of the fetal scrotum. Similar results were observed by other worker(1 and 12). In Blackface sheep (6) that an increases in fetal body weight, testes mass during 50 and 75days of age.The large increases in rate of fetal growth and testes mass were reported after 110 days . The appearance of the testes in the inguinal ring and/ or scrotum (table 2). These observations are similar to those reported by Beardow (13) and Chenowethand Kastlic (14). Spermatogonium cells and a few leydig cells present in seminiferous tubules (Fig. 1) The different types of germinal cells increased in number and size with an increasing in the testes mass and the presence of leydig cells at early stages of fetal development, it indicated that the testes could produce testosterone which is important for the regulation of the testicular descent and increasing in the size and number of differene spermatogunium cells and somatic cells . The development and function of the testes in sheep fetuses are known to be critically dependent on the circulating fetal gonadotrophin concentrations (2).Short(4) reported that the increases in testosterone at day 50 conicides with aperiod of enhanced CNS sensitivity to androgen.When the testes in the scrotum, the histological sections describes the increases in number and size of the leydig cells (Fig.5 and 6). At the same time the presence of different types of spermatogonium cells and spermatocytes cells with

a few metamorphosis spermatid indicated (Fig.6). While (Fig.7) shows the increases in number and development of different types of metamorphosis spermatid in seminiferous tubules, no mature spermatid appear in the sections. Nathanielsz (18) recorded that the first appearance of spermatozoa in testes at about 34 days after birth in sheep .This indicates that the formation of the spermatid seen before birth with other germinal cells and leydig cells are present during fetal life. Masek (15) and Kim (16) repoorted an increase in fetal testosterone between days 30-90 of gestation coincides with a period of reproductive function. Kosut (17) reported that the injection of testosterone during gestation can advance the time of fetal testes developing. Testosterone concentrations were generally higher in fetuses ,reflect the age of fetus and associated stages of development and steroidogenic capacity(6). It was concluded from this study the presences of leydig cells before day 50 indicated that fetal testes could be producing its own androgen which important in increasing in fetal testes mass due to increasing in size and number in different spermatogunium and spermatocytes cells with appearance of metamorphosis spermatid.

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