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CONTROL THE ONSET OF PUBERTY BY APPLICATION OF GONADOTROPIN RELEASING HORMONE (GNRH) IN IRAQI LAMB RAMS

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Abstract: This study is design to investigate further the effect of two GnRH level treatments to onset of lamb-ram puberty and to minimize the incidence of sexual maturity age. Eighteen lamb-rams of 4-5 month of age with no previous history, body wt. around 14-17 kg, scrotal circumference around 8-10 cm, was conducted on this study which started from early November 2019 to the end of March 2020, at the animal yard of the Veterinary MedicineCollege - University of Baghdad- Al-Amerya state. Lamb-rams randomly divided into three groups; 1) Group (A) was treated with GESTAR (GnRH 0.00042g) 2ml /lamb/48 hs. 2) Group (B) was treated with GESTAR(GnRH 0.00042g)1ml/lamb/48 hs. 3) Group(C) was received nothing and stay as control group. All experimental lambs were received an equal quantities of food as 2kg of straw and 1 kg of oat and barely (grained). Testicular circumference, body wt. and hormonal analysis determined every 15 days. Results showed that; Testicular wt, of group of 2ml GnRH(A) 138.5±1.5g,group of 1ml GnRH(B) 190.0± 10.0g, and for control group(C) 96.5± 6.5g, Sc was of A) 25.5±0.5 cm, B) 29.75±0.75cm and C) 21.5±0.5, Testicular length was of A) 9.0±0.0 cm, B) 10.75±0.25cm and C) 7.25±0.25cm, testicular diameter was of A)5.05±0.05cm., B) 5.55±.05cm and C) 3.5±0.1cm. testicular size was of A)140.0±0.0ml, B) 195.0±10.0ml and C)100.0±5.0ml, animal wt. was of A)37.5±1.5kg, B)42.0±3.0kg and C)36.0±1.0, testosterone level was of A)1.3±0.52ng/ml, B)2.12±0.74ng/ml and C)0.271±0.18ng/ml, estrogen level was of A) 5.81±0.38pg/ml, B) 11.63±3.7pg/ml and C)5.15±0.22pg/ml, volume of semen was of A) 0.675±0.04ml, B)0.90±0.04ml and C)0.60±0.04ml, mass motility was of A)75.0±2.04% , B)82.5±1.44% and C)65.0±2.04% ,individual motility was of A)78.75±1.25% ,B)86.25±1.25 and C)67.5±1.44 ,viability was of A) 81.0±1.29% ,B) 89.0±0.91% and C)70.0±1.82 and normal sperm was of A)80.5±2.1% ,B) 93.0±0.57% and C)71.25±1.54% . In conclusion 1ml Gestar (0,00042gm GnRH) is better in controlling and induction of puberty and sexual maturity in lamb-ram at 120 day treatment.

Keywords; lamb-ram, scrotal circumference, GnRH, testosterone, estrogen, electrical-ejaculator, urethral process

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INTRODUCTION

The complicated mechanisms which underlying gonads development and sexual maturity in domestic animals reflects the complexity to define accurately onset of puberty particularly in species with seasonal reproduction activity (Emsen, 2005). The interaction between body weight, testis growth, testosterone secretion (Martinez, et al. 2012), and sperm production, especially during the pre-pubertal stage, is the key factors influencing puberty (Souza, et al., 2010). It has been established that puberty in male lambs depends on genetic origin (Kridli, et al., 2006), nutrition (Foster, et al., 1988; Khalifa, et al., 2013), birth date, and photoperiod length (Foster, et al., 1988). However, in sheep, puberty seems to be more dependent on body weight considered as the determinant factor (Dyrmundsson and Lees, 1972; Haynes and Schanbacher, 1983). Different indicators are commonly used to determine the onset of puberty; nevertheless, the solid yardsticks are the increased testicular size simultaneously to the first presence of spermatozoa in seminiferous tubules, epididymis, and ejaculates (Kridli, et al., 2006; Preyorious and Marincowitz, 1968). Alternatively, puberty is determined on the basis of testicular growth (Salisbury, et al., 1978), testosterone concentration (Kridli, et al., 2006). Separation of the penis and urethral process from the prepuce (Madani, et al., 1989).

Increase in the temperature of the environment effects on the function of reproductive in the male by changing of spermatogenesis and reducing semen quality, this affects leading to decreased male fertility (Curtis 1983; Kashef 2012). Mickelsen et al. (1981) showed that season and breed had more effect on scrotal circumference than did body weight. Ram sexual activities can be demonstrated by many factors, as seasons, genetics, breed differences, hormonal pictures, post-weaning management, nutrition and temperature (Mickelsen et al., 1982). Daylight is the principal environmental factor affecting sheep reproduction (Chemineau et al., 1992). Awassi sheep are considered to have effected by breeding season, related mainly to food (Epstein. 1982). Semen quality and scrotal circumference (Sc) of Awassi rams appear to be better in the autumn (Gundogan, 2006). Effect of season, to change in semen quality and libido in ram, but males are not quietly influenced by photoperiod to the same degree as females do, in which males continue to produce fertile sperms and exhibit sexual behavior throughout the year (Langford et al., 1998; Salhab et al., 2003).

Spermatogenesis is a process that occurs in ST of the testis (Cheng, et al., 2010). Spermatogonia cells are renew itself by mitosis, meiosis (I, II) and participate to the establishment of haploid spermatids from diploid spermatocytes. During the procedure of spermiogenesis, spermatids under the effect of maturation and transformed to functional state (Yoshida,et al.,2007).Sperms which are liberated by spermiation after intercellular break down of the bridges attaching spermatids to Sertoli cells (Neto, et

al.,2016). Intra and extra-testicular regulatory hormones lead to release of Follicle Stimulation Hormone from anterior lope of pituitary gland and T from interstitial cell are the main mechanisms of spermatogenesis (Walker, 2009). Sertoli cells control the milieu within (ST) to development of germ cells to sperm. Spermatogenesis is a process ordered and controlled by action of LH on Leydig cells and FSH on Sertoli cells (Walker. 2009).

Function and development of the testis demand a complicated group of cell differentiation, proliferation, and communication in both fetal and postnatal life. This cascade beginning is reliant on the activity of Sertoli cells (Karl, et al., 1998). Sertoli cells control formation of ST, (BTB), peritubular myoid cell function, Leydig cell development and survival of germ cell development (Rebourcet, et al., 2014).

Orth, et al. (1988) mentioned that; reducing in Sertoli cell proliferation of new born could be causes a similar change in Sertoli cell and decrease in the number of spermatid in adulthood. Sertoli cell is a kind of sustentacular cell or a somatic "nurse" cell of testis that is part of ST and assistances in the procedure of spermatogenesis, it is regulated by (FSH) of Adenohypophysis, and it has FSH receptor (Rato, et al.,2012). Sertoli cells (mother cells) act like phagocytes which consumed the cytoplasm residue during spermatogenesis. Cells changing location in seminiferous tubule (ST) from base to lumen by direct changes of the lateral margins in the Sertoli cells (Rato, et al., 2012). Sertoli cells as mother cells secreted many hormones and factors which are; Anti-Mullerian hormone (AMH) during early stages of fetal life, Inhibin, Activins, Androgen binding protein (ABP), Estradiol ,Glial cell line-derived neurotropic factor (GDNF) and Transferrin (O'Donnell,et al.,2011).

Leydig cells or interstitial cells are percent near to the ST in the testis. They produced T in the existence of luteinizing hormone (LH). Leydig cells are polyhedral in shape, have a large prominent nucleus, with eosinophilic cytoplasm, numerous lipid-filled vesicles, large amounts of smooth endoplasmic reticulum (SER) and several mitochondria (Al-Agha and Axiotis, 2007). Upon this signs and charactical picture of interstitial cell within the testes no other cells appeared like that, making identification relatively easy (Ramnani and Dharam 2011). Leydig cells release the steroids hormone androgens (19 carbon atoms), as testosterone, dehydroepiandrosterone (DHEA) and androstenedione, these cells stimulated by (LH). LH increases the activity of cholesterol desmolase (the enzyme involved in converting cholesterol to pregnenolone) lead to manufacture and excretion of testosterone by Leydig cells. The Prolactin hormone (PRL) improved response of Leydig cells to Luteinizing hormone by improved the number of Luteinizing hormone receptors which, expressed on Leydig cells (Svechnikov, et al., 2010).

Materials and Methods

Eighteen Iraqi ram lambs with no history aged between 4 to5 months old (Fig.1), body wt. about 14-17Kg, its scrotal circumference around 8-10 centimeters where subjected to this study. All lambs are kept under same conditions and clinically examined, which included general evaluation (body condition inspection, respiratory, circulatory, digestive and musculoskeletal systems evaluation as possible), grossly examination of genitalia (inspection and palpation of the scrotum, testes, epididymis, spermatic cords, prepuce and penis as possible). All animal were injected with antibiotics as a profelective treatment (Enrofloxacin 10% and Tylosine 20% injection), multivitamin (Oligo-vit) for four days, all lambs were had an anti-parasitic treatment therapy against ecto- and endo-parasites (Rafoxanide and Ivermactin), vaccinated against clostridia infection. The animals were randomly divided into three groups (six lamb each), group A, B and C, group A is received 2 ml GnRH (Gestar) equivalent to (84µg) every 48 hs, while group B received 1ml (42µg) of GnRH (48 hs apart), while C group was received nothing and stay as control group. All experimental lambs were received an equal quantities of food all experiment period which consist of 2kg of straw and 1 kg of oat and barely (grained).

This study was designed to control the onset of puberty in Iraqi lamb rams by two different doses of GnRH groups with control group achievement in relation to Iraqi environments which controlled the testicular parameters, body wt. and hormonal analysis. Study was started from early November 2019 to the end of March 2020, at the animal yard of the Veterinary Medicine College - University of Baghdad-Alamirreia state.

Figure 1 :The experimental lamb-rams

Endocrinological evaluations

Blood samples (5 ml) were collected every 15 days from the jugular. Serum was separated by centrifugation at 3000 rpm for 10 minutes then directly transferred to lab for analysis. The Testosterone, Estradiol and FSH levels were determined by solid phase radioimmunoassay. All results were recorded.

Morphological evaluations

A biometry was performed in the testicular region as mentioned by (Matos Cap.et al.,1992). Scrotal circumference (Sc) was obtained by forcing both testicles to descend completely into the scrotum, with the aid of a flexible measuring tape placed at the maximum transverse diameter encountered scrotal sac (Bielli A. et al.,2000) performed every 15 days, body weight, penis and urethral process development. By the end of experiment two animals were slaughtered from each group to estimate testicular parameter as testicular length, diameter by digital vernia and testicular volume by water displacement Figure (2,3).

Figure 2. Scrotal circumference

Figure 3. Testicular diameter

1 Semen collection

Semen was collected by electro-ejaculator apparatus (fig 4) with rectal probe of 3-5 volts was inserted through the rectum for electric stimulation, semen samplewere kept in a thermo-flask at 37ºC and transferred to the laboratory for evaluation within 1hour after collection (Matshaba B. 2010).

Figure 4.Intromission of the Electro-ejaculator apparatus

Figure 5.Semen collection

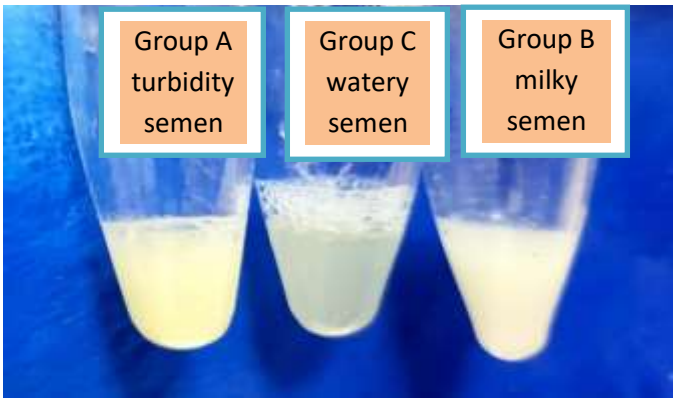
Semen evaluation

Semen sample were evaluated macroscopically as semen volume and color, microscopically for sperm concentration, motility, morphology and viability.

Macroscopic semen evaluation

Macroscopic semen evaluation was included physical as color, volume and consistency on 10 ml graduated falcon tubes, all results were recorded (Figure 6).

Fig. (3.6): Show Macroscopical seminal evaluation



Microscopic semen evaluation

Microscopic semen evaluation is includedmass motility which performed by putting one drop of fresh semen on slide, examined under microscope, while individual motility was of performed after dilution of semen by adding 0.1 fresh semen with 0.9 Tri-sodium citrate (2,9%), final results were recorded as total progressivepercentage , non-progressive static, slow, medium and rapid motility, sperm abnormalities and sperm viability were determined by eosin-Nigrosine stain slide with a diluted rate of 1:4 eosin-Nigrosine (5µl of raw semen and 20µl eosin-Nigrosine), live or dead sperm(Fig.7,8)

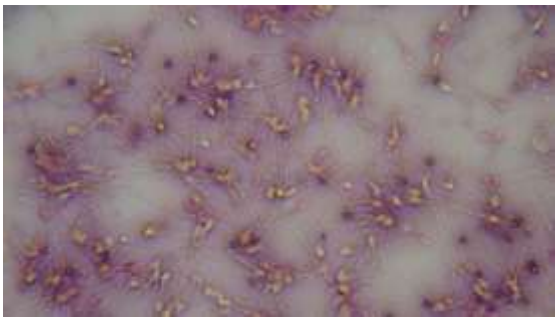


Figure 7:Eosin-Nigrosine stained seminal sample of 2cc(84µg GnRH), less alive &account sperm

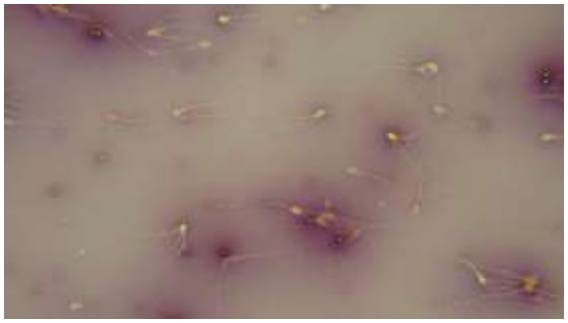


Figure 8:Eosin - Nigrosine stained seminal sample of 1cc (42µg GnRH) shown high alive sperm concentration

The result

The results of this study explained the effect of the GnRH injection on controlling of lamb-ram puberty. 1-GnRH injection of one cc (42 µg) on 48 hs apart on body wt. gave more effect than two cc (84µg) on

the same period as compared with that of the control under same experimental condition, one cc injected animals showed a significant ($p< 0.05$) increased body wt than the other group even the control one, as shown in table 1

Table 1: Effect of Hormone injection (GnRH) on animal weight

Period (time)	Mean ± SE of animal weight (Kg)			LSD value
	Group A 2ml GnRH(84µg)	Group B 1ml GnRH(42µg)	Group C control	
10/11	15.67 ±0.49	16.08 ±0.45	15.91 ±0.43	1.394 NS
25/11	17.41 ±0.66	18.33 ±0.72	16.91 ±0.55	1.965 NS
10/12	9.33 ±0.83 ab	21.33 ±1.32 a	18.16 ±0.58 b	2.912 *
25/12	0.25 ±0.76 ab	23.00 ±1.80 a	18.83 ±0.64 b	3.590 *
10/1	1.33 ±1.02 ab	25.25 ±2.47 a	20.00 ±0.89 b	4.914 *
25/1	22.50 ±1.29	26.91 ±3.05	21.67 ±1.26	6.169 NS
10/2	23.91 ±1.21	27.66 ±2.57	23.16 ±1.27	5.451 NS
25/2	25.66 ±0.84	28.91 ±2.57	24.67 ±1.25	5.190 NS
10/3	28.00 ±1.09	32.58 ±2.67	27.33 ±1.67	5.802 NS
25/3	1.67 ±0.98 b	36.83 ±2.45 a	32.33 ±1.21 ab	5.058 *
Means having with the different letters in same row differed significantly.* (P≤0.05).				

2- GnRH injection of one cc (42 µg) on 48 hs apart on Scrotal circumference (Sc) gave more effect than two cc (84µg) on the same period as compared with that of the control under same experimental condition, one cc injected animals showed a significant ($p< 0.05$) increased Sc than the other group even the control one,as shown in table (2).

Table 2: Effect of Hormone injection (GnRH) on Scrotum circumference

Period (time)	Mean ± SE ofScrotum circumference (cm)			LSD value
	Group A 2ml GnRH(84µg)	Group B 1ml GnRH(42µg)	Group C control	
10/11	8.75 ±0.25	9.25 ±0.31	9.16 ±0.33	0.903 NS
25/11	10.91 ±0.27 ab	11.50 ±0.36 a	10.08 ±0.32 b	0.975 *
10/12	11.83 ±0.16 b	12.83 ±0.35 a	11.25 ±0.33 b	0.901 *
25/12	12.33 ±0.24 b	14.58 ±0.78 a	12.41 ±0.56 b	1.733 *
10/1	12.91 ±0.30 b	15.91 ±1.17 a	13.16 ±0.65 b	2.404 *
25/1	13.75 ±0.40 b	17.33 ±1.70 a	14.00 ±0.69 b	3.282 *
10/2	14.16 ±0.53 b	18.83 ±1.81 a	15.08 ±0.75 b	3.536 *
25/2	15.75 ±0.70 b	20.91 ±1.72 a	15.91 ±0.78 b	3.520 *
10/3	17.41 ±1.00 b	22.16 ±1.57 a	17.08 ±0.86 b	3.584 *
25/3	18.83 ±1.32 b	24.33 ±1.35 a	17.91 ±1.07 b	3.797 *
ans having with the different letters in same row differed significantly.* (P≤0.05).				

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3-The effect of GnRH injection on testicular parameter in relation to Sc and body wt after animal slaughter showed that; one GnRH (42µg) injection significantly (P≤0.05) increased more than the other level evens the control one as shown in table (3).

Table 3: Effect of Hormone injection (GnRH) on testicular parameters in relationto Sc and animal wt.

Group	Animal wt.\Kg	Scrotum cumference/ cm	Testicular size\ml	Testicular wt.\g	Testicular ength\cm	Testicular iameter\cm
Group A 2ml GnRH(84µg)	.50 ±1.50	.50 ±0.50 b	0.00 ±0.00 b	38.50 ±1.50 b	.00 ±0.00 b	.05 ±0.05 b
Group B 1ml GnRH(42µg)	.00 ±3.00	.75 ±0.75 a	5.00 ±10.00 a	0.00 ±10.00 a	.75 ±0.25 a	.55 ±0.05 a
roup CControl	.00 ±1.00	.50 ±0.50 c	0.00 ±5.00 c	6.50 ±6.50 c	.25 ±0.25 c	.50 ±0.10 c
LSD value	.094 NS	2.678 *	29.052 *	31.236 *	0.918 *	0.318 *

ans having with the different letters in same column differed significantly.* (P≤0.05).

4- Effect of GnRH injection on testosterone level

Testosterone level increased in all lambs with age, there were significant($p<0.05$) differences in their means in the different groups with age.The animals in group B of 1ml GnRH injection(42µg) on 48 hs apart showed a significantly($p<0.05$) higher testosterone concentration than group A of 2 ml(84µg) on the same period as compared with that of group C the control one under same experimental condition, as shown in table (4).

Table 4: Effect of Hormone injection (GnRH) on Testosterone concentration

Period (time)	Mean ± SE of Testosterone level (ng/ml)			LSD value
	Group A 2ml GnRH(84µg)	Group B 1ml GnRH(42µg)	roup C control	
10/11	0.013 ±0.001	0.021 ±0.007	0.045 ±0.03	0.055 NS
25/11	0.033 ±0.012 b	0.107 ±0.031 a	.022 ±0.007 b	0.060 *
10/12	0.019 ±0.002 b	0.411 ±0.194 a	.025 ±0.008 b	0.338 *
25/12	0.032 ±0.009 b	0.524 ±0.270 a	.033 ±0.012 b	0.472 *
10/1	0.039 ±0.012 b	0.466 ±0.088 a	0.145 ±0.08 b	0.213 *
25/1	0.051 ±0.011 b	1.66 ±0.62 a	0.282 ±0.16 b	1.130 *
10/2	0.716 ±0.212 b	2.033 ±0.56 a	0.165 ±0.11 b	1.068 *
25/2	2.87 ±0.32 b	4.36 ±0.68 a	0.700 ±0.39 c	1.494 *
10/3	0.250 ±0.083 b	1.61 ±0.54 a	0.460 ±0.25 b	1.050 *
25/3	1.30 ±0.52 ab	2.12 ±0.74 a	0.271 ±0.18 b	1.627 *

ans having with the different letters in same row differed significantly. * (P≤0.05).

5- Effect of GnRH injection on estrogen concentration

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GnRH injection effect the Estrogen concentration which shown as no significant increase at the first two months with one ml (42µg), but a significantly (P≤0.05) increased result appeared at the following period more than the other level even the control one as shown in table(5).

Table 5: Effect of Hormone injection (GnRH) on Estrogen concentration

Period (time)	Mean ± SE of Estrogen level (pg./ml)			LSD value
	Group A 2ml GnRH(84µg)	Group B 1ml GnRH(42µg)	Group C control	
10/11	4.48 ±0.16	4.75 ±0.27	6.48 ±1.88	3.329 NS
25/11	4.76 ±0.31	5.35 ±0.34	5.01 ±0.45	1.134 NS
10/12	4.55 ±0.19	7.28 ±2.36	4.58 ±0.10	4.141 NS
25/12	4.68 ±0.14	6.10 ±0.81	4.71 ±0.16	1.464 NS
10/1	4.76 ±0.18	7.31 ±1.75	4.68 ±0.15	3.089 NS
25/1	4.83 ±0.14 b	10.13 ±2.65 a	4.65 ±0.15 b	4.631 *
10/2	5.00 ±0.26	6.46 ±1.01	4.73 ±0.11	1.832 NS
25/2	5.15 ±0.90 ab	6.50 ±0.91 a	4.76 ±0.11 b	1.629 *
10/3	6.00 ±0.90 ab	9.26 ±2.09 a	4.95 ±0.12 b	3.826 *
25/3	5.81 ±0.38	11.63 ±3.70	5.15 ±0.22	6.489 NS
Means having with the different letters in same row differed significantly. * (P≤0.05).				

6- Effect of GnRH injection on FSH level

Results of the effect of GnRH injection upon two levels showed no significant changes concerning the FSH level even the control group include all period of the experiment, this unchanged level may be due to physiological effect of FSH on the activity of Sertoli and Leydig cells table (6).

Table 6: Effect of Hormone injection (GnRH) on Follicular Stimulating Hormone concentration

Period (time)	Mean ± SE of FSH level (MIU/ml)			LSD value
	Group A 2ml GnRH(84µg)	Group B 1ml GnRH(42µg)	Group C control	
10/11	0.085 ±0.002	0.085 ±0.003	0.086 ±0.002	0.008 NS
25/11	0.087 ±0.002	0.085 ±0.002	0.087 ±0.002	0.0058 NS
10/12	0.087 ±0.001	0.086 ±0.001	0.087 ±0.002	0.0048 NS
25/12	0.086 ±0.003	0.089 ±0.001	0.087 ±0.002	0.0069 NS
10/1	0.085 ±0.002	0.088 ±0.002	0.087 ±0.002	0.0065 NS
25/1	0.089 ±0.001	0.090 ±0.001	0.088 ±0.002	0.0054 NS
10/2	0.090 ±0.002	0.088 ±0.002	0.090 ±0.002	0.0065 NS
25/2	0.090 ±0.002	0.089 ±0.002	0.090 ±0.002	0.0059 NS

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10/3	0.091 ±0.001	0.087 ±0.002	0.091 ±0.002	0.0059 NS
25/3	0.093 ±0.001	0.089 ±0.002	0.091 ±0.002	0.0063 NS
n-Significantly.				

7. Effect of GnRH injection on semen consistency

The semen consistency were significantly($p<0.05$) better in group B of 1ml GnRH injection(42µg) on 48 hs apartthan group A of 2 ml(84µg) on the same period as compared with that of group C the control one under same experimental condition in all collection time, as shown in tab. (7).

Table 7: Effect of hormone injection (GnRH) in relationship to electrical stimulation on semen quality

Animal	Nature of semen in collection	Nature of semen in collection	Nature of semen in collection
G. A 2ml GnRH(84µ			
1	Milky	Milky	Milky
2	Milky	Milky	Milky
3	Turbidity	Turbidity	Turbidity
4	Milky	Turbidity	Milky
G. B 1ml GnRH(42µ			
1	Milky	Milky	Milky
2	Milky	Milky	Milky
3	Milky	Milky	Milky
4	Milky	Milky	Milky
G. C control			
1	Turbidity	Turbidity	Turbidity
2	Watery	Watery	Watery
3	Milky	Turbidity	Milky
4	Turbidity	Turbidity	Watery

8. Effect of GnRH injection on semen evaluation

The semen volume, mass motility, individual motility, viability and normal sperm were significantly($p<0.05$) higher percentages in group B of 1ml GnRH injection on 48 hs apartthan group A of 2 ml on the same period as compared with that of group C the control one under same experimental condition,and higher percentages in group A compared with group C,however the abnormal were significantly($p<0.05$) higher percentage in control group than the two group A and B of GnRH injection, as shown in tab. (8).

Table 8: Effect of hormone injection (GnRH) on semen evaluation

Group	olume/ml	ss motility (%)	ndividual otility (%)	bility (%)	ormal rm (%)	Abnormal sperm			
						ached head (%)	uble head (%)	Coiled Tail%	Protoplasmic droplet(%)
up A 2ml GnRH(84µg)	75 ±0.04 b	0 ±2.04 b	.75 ±1.25 b	0 ±1.29 b	0 ±2.10 b	1.62 ±0.24 b	875 ±0.12 a	25 ±0.32 b	13.75 ±1.49 a
up B 1ml GnRH(42µg)	0 ±0.04 a	0 ±1.44 a	.25 ±1.25 a	0 ±0.91 a	0 ±0.57 a	0.625 ±0.12 b	375 ±0.12 b	50 ±0.28 b	2.50 ±0.28 b
Group C Control	0 ±0.04 b	0 ±2.04 c	.50 ±1.44 c	0 ±1.82 c	5±1.54 c	4.50 ±0.64 a	875 ±0.12 a	62±0.37 a	15.75 ±1.11 a
LSD value	0.138 *	6.961 *	4.215 *	.461 *	937 *	1.292 *	0.399 *	1.058 *	3.476 *

ans having with the different letters in same column differed significantly. * (P≤0.05).

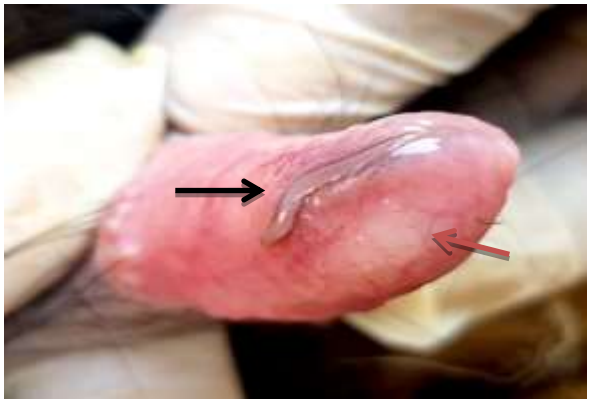

Figure 9. Lamb-ram glans penis (Galea glandis) penis (Galea glandis) Red arrow with an undeveloped Urethral process (Black arrow) Juvenile age.

Figure 10. Lamb-ram glans red arrow with a slightly developed urethral process (Black arrow). One month GnRH treatment

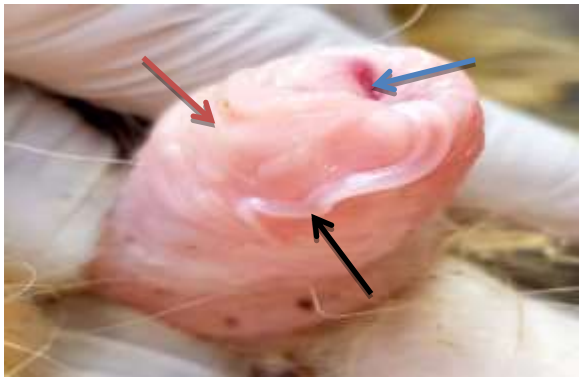

Figure 11.Lamb-ram glans penis (Galea glandis) Red arrow with a slight developed Urethral (Black arrow), after 45 days GnRH treatment

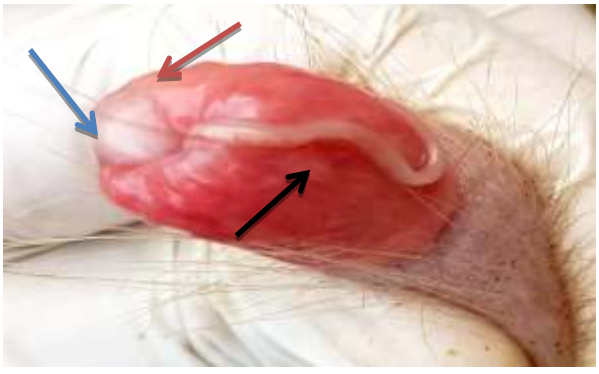

Figure 12. Lamb-ram glans penis (Galea glandis) red arrow with a slightly developed urethral process (black arrow). 45 days GnRH treatment.

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Blue arrow seems to be the external urethral orifice

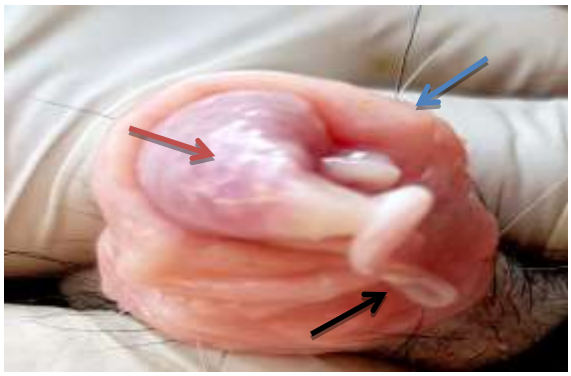

Figure 13.Lamb-ram glans penis (Galea glandis)
Red arrow with a well-developed Urethral process
(Black arrow), after three months GnRH treatment
With a beginning to separate from prepuce, blue
Arrow seems to be the prepuce.

Blue arrow seems to be the external urethral orifice

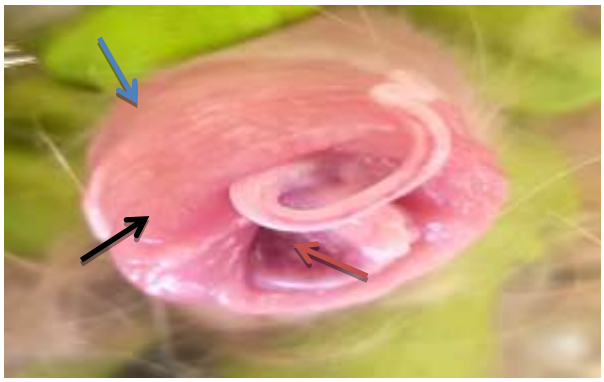

Figure 14. Lamb-ram glans penis (Galea glandis)
red arrow with a slightly developed urethral process
(black arrow). Two months GnRH treatment.
Semi-complete urethral process separation
Blue arrow seems to be the prepuce.

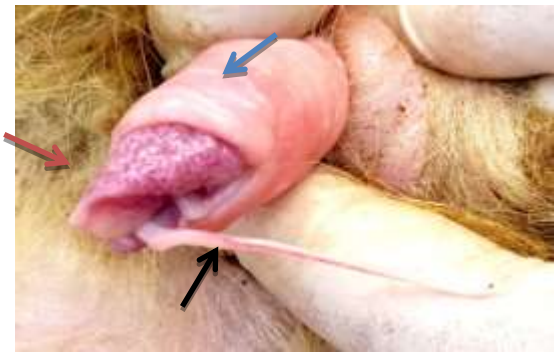

Figure 15. Complete urethral separation of lamb ram(Black arrow) as a sign of puberty, red arrow denoted the Glans penis, blue arrow is the prepuce

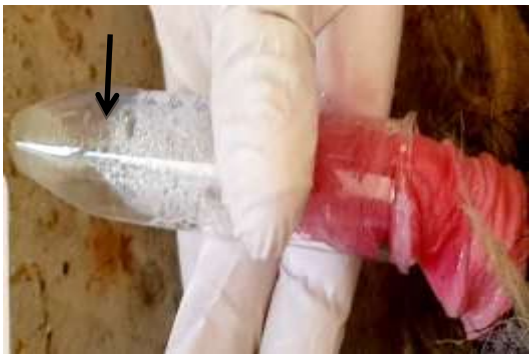

Figure 16. Ejaculated seminal fluid (black arrow) after Electrical ejaculator stimulation after more than three months GnRH treatment.

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Figure 17 Green star denoted the normal ram semen sample (1cc GnRH injection). Orange star denoted the 2ccGnRH injection (slight turbid and with less consistency). Black star denoted the semen of the control group with no hormone treatment (watery in consistency).

Figure 18 . Three testicular samples of Lamb-ram after slaughtered by the end of the experiment A) Testicule of 2 cc (84µg) GnRH treatment lamb-ram, slightly well-developed. B) Testicular sample of 1cc (42µg) GnRH well-developed and size. C) Testicle of the control.

THE DISCUSSION

This study was design to investigate further the effect of the GnRH on minimizing the time of onset of puberty in lamb-ram under Iraqi circumstances which is mainly prolonged due to these circumstances, in which; body weight increased in all lambs with age but there were a significant ($p<0.05$) differences in their means in groups, lamb-rams of group B with 1ml GnRH injection(42µg) on 48 hs apart showed a higher body wt. than group A of 2 ml(84µg) on the same period as compared with that of group C the control one under same experimental condition. This is approved by Ramzi, (2010) and Al-Musawey, (2009) in gaining more body wt. as sheep continued in growth up to puberty then sexual maturity. The GnRH effect to testosterone level might be the cause or the reason to increase the appetite and enhance digestion, which leads to increase conversion of nutrients like amino acid, glucose and volatile fatty acids (Tamir et al., 2001). Amino acid could improve the secretion of growth hormone from the anterior lobe of pituitary gland (Defali and Bourne, 1998).

The results of this study showed that; body wt. are very important factors to determine the age of reaching puberty, these results were agreed with (Cui et al.,2003). Lamb-rams in group B showed signs of puberty when reached a body wt. of (27.66±2.57kg) at 7th month of age, while animals of group A and C reached puberty when its body wt. (28.00±1.09kg) and (27.33±1.67kg) respectively at 9th month

of age .These results were agreed with the result that found by Al-Molla and Kridli, (2003) in Awassi lambs which reached puberty at nine months of age, and proved with the result that found by Cui et al.(2003) lamb rams reached puberty between 8th and 9th month of age. Evans et al.(2012) found that; time of pubertal age incidence will be diminished eight weeks lesser than the normal time of reaching puberty which is about 28 week when induced by GnRH stimulator to the hypothalamus to secrete GnRH and agreed with the result of Bearden and Fuquay,(1997) that Awassi lambs were reached puberty when sheep reach 40 to 50% of their mature body wt. (32±4kg).

This increase result in body weight is accompanied with an increased testicular size (Gundogan and Serteser 2005; Mudhaffar and Saad, 2012) which mainly lead to positive changes in scrotal circumference. In this study increased Sc is mainly followed the GnRH treatment which lead to increased total body weight, and this affected the testicular size positively. This is agreed with that mentioned by Zamiri and Khodaei (2005) that the testicular size and its circumference is a standard of fertility and reproductive ability in ram. Salhab, et al, (2001) also agreed with this in which; Sc has been described as the best criterion of male sexual development.Michelle et al, (2009) and Cheng et al, (2010) found that; spermatogenesis is an organized process throughout the life of the male, it does not occur simultaneously in all seminiferous tubules but rather as wave-like sequences of maturation, referred to as cycle of seminiferous epithelium which leads in turn to more testicular activity and more size. Results showed that; testicular measurement and Sc are more correlated with body wt. of growing rams than the age, this result were agreed with Salhab et al. (2001) which found similar result but disagreed with Toe et al.(1994), that age is one of the major contributing factors to differences in Sc and semen characteristics.

Results showed that; there was a positive correlation of GnRH treatment on testicular size, Sc, and testicles weight, 1 ml GnRH treatment gives more testicular weight than the 2 ml or even the control. This is agreed with the result found by Toe et al (2000) that the testicular size may be a useful selection criterion for improving reproductive capacity in sheep. Bilgin et al, (2004) also mentioned and approved that; gonadotropins is controlled the testicular size of young and adult ram which act as main parameter for reproductive efficiency.

Results showed that; all parameters which obtained from group B lamb-ram are significantly more positive than the group A (2ml GnRH) or even the control group, and this might be related to hormone and its receptors. Receptors are created, or expressed, from instructions in the DNA of the cell, and they can be increased, or upregulated, when the signal is weak, or decreased, or downregulated, when it is strong. Their level can also be up or down regulated by modulation of systems that degrade receptors

when they are no longer required by the cell. This is agreed with those explained by Zaliauskiene et al. (2016) that its related to the up-regulation and down-regulation of the hormone receptors phenomenon. Down-regulation of receptors can occur when receptors have been chronically exposed to an excessive amount of a causative factor, either from endogenous mediators or from exogenous drugs, this results in factor-induced desensitization or internalization of that receptor; this is typically seen in animal hormone receptors. Up-regulation of receptors, on the other hand, can result in super-sensitized cells especially after repeated exposure to a causative factor (hormone) antagonistic drug or prolonged absence of the factor.

The results of the present work showed that; body wt. of lamb rams were positive correlated with testes length, Sc, testicular weight, testicular volume and testicular diameter. These results are in agreement with Hamdon,(2005) in which; age and body wt. of lamb-rams were positively correlated with testes length, Sc and testicular volume, and agreed with Salhab et al.(2001) mentioned that the age and the body wt. of Awassi lamb rams were positive relationship with testicular measurements. Hassan et al. (2009), Ibrahim et al. (2012) and Omar (2016) mentioned that testicular length could be a useful estimate of testicular growth because of its high correlations with the other testicular measurement.

Upon hormonal analysis, this study is looked for the testosterone level as a guide for puberty development and sexual maturity incidence, in which; T level is continuously increased as the age of lamb-ram is increased (positively), but T level of 1ml lamb-ram treated showed more elevated and significantly increased level than the 2ml treated lamb or even the control one. This increased level could be either for up or down-regulation phenomenon discussed later or for the direct effect of the exogenous gonadotropin (GnRH) on the anterior pituitary lobe to secrete LH which in turned affect and stimulate the interstitial cells (Leydig cells) to secrete T for spermatogenesis and secondary sex characters. This fact is approved by Charles et al (2002) and Stellflug et al (2004) that; higher T level in ram having great Sc and testicular weight. Boukenaoui et al, (2012) found that; testicular growth is related to increasing of T receptors which in turn lead to high T level; these receptors are under FSH control which released by GnRH injection. Saeed and Zaid (2019) agreed with this result that; T hormone could be used as an indicator for puberty in lamb-ram.High T level agreed with Miller et al. (1989), serum T levels were increased gradually in ram lambs from 20 to 30 week of age and agreed with Kridli and Al-Yacoub, (2006) in which T level was positively correlated with body wt. and Sc, and agreed with Rajak et al. (2014), T was low at 30- 180 days of age and rise to at 200 days of age, after that, there was gradual increased with age to reach sexual maturity, this elevation of testosterone level with advancing of age considered as indicator for the increasing of testicular activity.

Estrogen level analysis showed a picture of fluctuation degree mainly with the group B which received 1ml GnRH treatment more than the A group that received 2ml GnRH treatment or even the C or control group, another thing; level of E2 at the first two month of the study stayed no significant. This fluctuation level is agreed with that of Sanford et al, (1982) which mainly related to a seasonal directional changes and to be 5% less than T level, E2 level in ram is associated with mating activity and libido, so that increased conversion of T to E2 could be the possible explanation.Cooke et al, (2017) explain this unstable E2 level in that; Estrogens have historically been associated with female reproduction, but work over the last two decades found that estrogens also regulate male reproductive and non-reproductive organs. Aromatase, which converts androgens to estrogens, is expressed in Leydig cells, seminiferous epithelium, and other male organs.Carreau, et al, (2008) by his initial work suggested that; FSH-stimulated Sertoli cells are primary sources of estrogen in immature males, while LH-stimulated Leydig cells are the primary source in adult testis, as they express more aromatase than adult Sertoli cells, for that; this might be the present source for this less E2 level.

FSH level upon the experiment duration and for all three group stayed low with no significant level, this is mainly due to the fact that; FSH is useful in the management of male fertility, this hormone with the LH and E2 are important for Leydig, Sertoli and other male system component. De- Kretser et al (1979) report that; elevated level of serum FSH with increasing severity of seminiferous epithelial distraction. Sheikh, et al,(2005) found that; in infertile males, higher concentration of FSH is considered to be reliable indicator of germinal epithelial damage, and was shown to be associated with azoospermia, and sever oligozoospermia.

Tilbrook and Clarke, (2001), Scott et al. (2004) mentioned that; T capable of inhibiting hypothalamic GnRH release without blocking GnRH direct action on the pituitary in the ram and human. In contrast's T'sjoen et al. (2005) and Ng et al. (2009) found that T aromatization to E2 can mediate inhibition of both pituitary GnRH action and hypothalamic GnRH release. Clarke et al. (1991) found that both steroidal (T, E2) and non-steroidal (inhibin) endocrine products of the testis regulate FSH secretion. All these previous results are explained our results by the lack of FSH level in lamb rams during study period.

Semen volume, mass and individual motility, viability and normal sperm were significantly ($p<0.05$) with higher percentage in group B than group A and C , and higher percentages with group A than group C. Abnormal sperm were significantly ($p<0.05$) higher percentage in control group (C) than the two groups of GnRH injection (A and B) in all collection time. These results may be due to variations in puberty and sexual maturity incidence which in turned related to in groups variations or differences in body wt., Sc, testicular parameter, T level and E2 level in different groups. These results were agreed by

Toe et al.(1994) were mentioned that semen characteristics related to Sc, and agreed by Ahmad and Noakes,(1995) found that testicular size being closely related to total sperm output, and agreed by Gordon(1997) were mentioned that a male with large symmetrical testes, free of defects is likely to produced semen of good quality ,and agreed by Gundogan and Serteser,(2005) reported significant correlation between testes volume and each of sperm concentration and sperm motility, and agreed by Salhab et al.(2001) were found that testis size is standard of fertility and reproduction ability, and agreed by Masanyi et al.(2000) were found that functional combination between testis release of T and sperm so that causes the T-dependent quality of spermatogenesis.

CONCLUSION

We can induce a puberty and sexual maturity in lamb-ram under Iraqi circumstances by GnRH treatment and a dose of 0.00042 gm. At 48 hours apart for 120 consecutive days with no need to increase the hormone dose or prolong the duration treatment time.

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