Veterinary Medical Journal – Giza
Faculty of Veterinary Medicine, Cairo University
(ISSN 1110 – 1423)
Accredited from National Authority for Quality Assurance and Accreditation
Giza, 12211 - Egypt

Gonadal steroids and leptin dynamics during estrous cycle of ewe: a correlative study

Essawy, G. S.[1]; Sawiress, F. A. R.[1]; Ogaly. H.A.[2]; Marei, W.F.[3]and Hendawy, A. Kh.[1]

[1].Department of Physiology, [2] Department of Biochemistry and Chemistry of Nutrition, and [3]. Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Egypt

ABSTRACT

Leptin, as a hormone, acts as a signal for the energy status and affects reproductive performance. The current study investigated dynamics of gonadal steroids and leptinduring estrous cycle in ewe. For this purpose synchronization of estrous cycle in ewes was accomplished by insertion of intravaginal sponge(Chronogest; Intervet Ltd.,) for 14 days followed by I/M injection of 300IU equine Chorionic Gonadotropin(Folligon; Intervet Ltd.,) for each ewe on day of sponge removal (day0), blood samples were collected during follicular and luteal phases for determination of leptin, estradioland progesterone hormones by ELISA technique.The obtained results showed fluctuation in levels of leptin, where a significant decrease was recorded during follicular phase and an increase during luteal phase.Estradiol levels were highest during 1st 2 days of the 17 and 18 days estrous cycle, reached lowest levels during days 7, 9, 11, 13 and 15of the 17 days estrous cycle.As for the 18 days estrous cycle the estradiol level remained low for one more day (17th day). Progesterone levels were lowest during first 3 days of the 17 days estrous cycle, while during the 18 days estrous cycle, low progesterone levels were detected up to the fifth day of the cycle; progesterone peaked at day 11of the 17 and 18 days estrous cycle.

Key words: estradiol, reproductive, estrous cycle, gonadal steroids, Progesterone levels

Introduction

Sheep is one of the most important farm animals in Egypt whereas they contribute to about 6% of total red meat production and also wool production as secondary product (Galal et al., 2005). Sheep are seasonally polyestrous animals; i.e. they come in estrus several times in a particular season "autumn and winter" (Aboul-Naga et al., 1987). Estrous cycle of ewe is classified into four stages: Proestrus, Estrus, Met-estrus and Di-estrus .Pro-estrus phase is the period immediately preceding estrus and it is characterized by follicular growth and regression of the corpus luteum of the previous cycle, it takes about 2 days (Goodman, 1994). Estrus phase (Heat) is considered as the period of sexual receptivity,it lasts 1-2 days, depending on the breed, stage of the breeding season, presence of a ram and age(Faber-Nys and Vernier, 1987).Sheep arespontaneousovulatoryanimals; ovulation occurs 24 to 30 hr. after the onset of estrus (Bindon et al., 1996). Met-estrus phase is the period after estrus and during which the remnants of the ovulated follicle transform into lutein cells, which are responsible for the formation of the corpus luteum (luteinization), the luteal structure in this phase is called corpus haemorrhagicum, and it takes about 2 days anddi-estrus phase is the longest phase of estrous cycle and characterized by presence of fully functional corpus luteum which secretes large amounts of progesterone and it lasts about 11-12 days (Keyes et al., 1983).During the follicular phase of the estrous cycle which takes about 2-3 days there is high level of estrogen hormone while during the luteal phase of the estrous cycle which is about 14-15days there is high level of progesterone hormone (Bjersing et al., 1972). Leptin hormone is the product of obese (Ob) gene, it is non-glycosylated 16 kDa protein which is synthesized and secreted predominantly by adipose tissues(Zhang et al., 1994)and it is also produced in low amount from a variety of other tissues, including placenta (Masuzaki et al., 1997), fetal cartilage (Hoggard et al., 1998), mammary tissue (Smith-Kirwin et al., 1998), skeletal muscle (Wang, 1998), pituitary tissue (Jin et al., 1999) and stomach (Sobhani et al.,2000).Concentration of leptin in blood is directly proportional to total amount of body fat and it may be found in the blood stream in its free form or bound with leptin-binding protein (Garcia et al., 2002). Leptin acts as a satiety signal playing an important role in regulation of feed intake, metabolism, energy balance, bone formation, hematopoiesis, immune function and reproduction (Houseknecht et al., 1998). Reproduction in mammals is very sensitive to availability of external environment, leptin plays a role in energy balance by preventing the fall in energy expenditure associated with energy restriction rather than to induce an increase above basal rates of energy expenditure (Doring et al.,

1998) and soleptin acts as a signal informing brain that metabolic store are adequate for the initiation of reproductive function, hencemalnutrition has deleterious effect on reproductive process including reduction inlibido, negative impact on pregnancy as increased fetal resorption and abortion(Scaramuzzi & Martin, 2008),the present study aimed to monitor leptin levels dynamics during the estrous cycle of ewe & correlate the variability in levels with those of gonadal steroids.

Materials and Methods

Animals:

The study was carried on nine apparently healthy ewes of 2 different breeds (5 Ossimi and 4 Rahmani),these animals, obtained from private market at Monofya governorate, were weighing 30-35 kg body weight and aging 8-10 months. The study was carried out from March 2014 till June 2014 then continued again from September till November 2014. The ewes were fedon maintenance ration and alfalfa twice daily. Clean, fresh water was available all times.The room where ewes were raised was well ventilated through natural ventilation and electric fan and ewes were exposed to natural lighting.

Experimental design:

After a period of acclimatization, ewes were confirmed to be non-pregnantbytrans-rectalultrasonographic examination.Synchronization of estrous cycle of ewes was accomplished by insertion of an intravaginal sponge (Chronogest; Intervet Ltd.,) for 14 days followed by I/M injection of 300 IU equine Chorionic Gonadotropin (Folligon; Intervet Ltd.,) for each ewe on the day of sponge removal (day0).

Blood sampling

Blood samples were collected at 9 a.m. after overnight fasting before administration of ration, from jugular vein using vacutainer containingEDTA starting at day1 after sponge removal and daily until day3 then every other day until day17or 18 then daily until day1,2 of the next cycle. Plasma was separated by centrifugation at 2000g for 10 minutes and kept at -20°C for hormonal assay.

Hormones assay:

Leptin,estradiol 17β and Progesterone levels were determined by Enzyme Linked Immune Sorbent Assay (ELISA) using kits purchased from "BioCheck, Inc." according to the methods of**Heymsfield et al. (1999),Ractliffe et al. (1988)** and**Tietz (1995)**respectively.

Statistical analysis:

All Data were subjected tostatistical analysisaccording to **Snedecor and Cochran (1980)**.Means were compared by the least significance difference test at 5% level of probability (t- test) at the different days of the estrous cycle; correlation studies between the measured parameters were adapted.

Results

Levels of leptin (ng/ml) along 17and 18 days estrous cycle in ewes:

Result tabulated in table (1) showed that levels of leptin were variable along 17 days estrous cycle, where the levels were lowest during 1st3 days of the estrous cycle and 1st2 days ofnext cyclethen levels started to increase significantly and progressively by the 5th day of the cycle, through the 7thand9th day and reached peak by the 11th day . The 15th day of the cycle showed significant decrease in leptin level compared to day 13 afterwards the levels of leptin decline significantly and progressively through the 17th dayof the estrous cycle and reached the lowest level during 2nddday of the next cycle.

Concerning of the 18 days estrous cycle, data illustrated in table (2) revealed that levels of leptin were variable along 18 days estrous cycle where the lowest values were recorded during 1st 3 days of the estrous cycle then levels started to increase significantly and progressively by the 7th and 9thdays of the cycle, reached peak by the 11th day of the cycle while day 17 of the cycle showed significant decrease in leptin level compared to day 15. Levels reached the lowest values during 18th day of thecycle and 1st day the next cycle.

Table (1): Levels of leptin (ng/ml) along 17 days estrous cycle in ewes:

	Day1	Day2	Day3	Day5	Day7	Day 9	Day11	Day13	Day15	Day17	Day1 of N.C	Day 2 of N.C	L.S.D =0.72
Mean	1.55	1.02	1.67	2.90	4.08	4.9	5.2	4.68	3.61	2.12	1.47	0.94	
S.E	±0.35	±0.29	±0.29	±0.41	± 0.4	± 0.15	± 0.07	± 0.15	± 0.23	± 0.16	± 0.15	± 0.16	

Data indicates mean ± S E, n = 6, p < 0.05, N.C. (Next Cycle).

Table (2): Levels of leptin (ng/ml) along 18 days estrous cycle in ewes:

	Day1	Day2	Day3	Day5	Day7	Day9	Day1	Day13	Day15	Day17	Day18	Da1 of N.C	Day2 of N.C	L.S.-D =1.66
Mean	0.83	1.23	1.5	2.43	4.27	4.73	4.73	4.5	3.5	1.63	1.1	1.06	1.5	
S.E	±0.17	±0.52	±0.56	±0.62	±0.94	±0.57	± 0.27	±0.40	± 0.96	±0.24	± 0.35	±0.41	±0.44	

Data indicates mean ± S.E, n = 3, p < 0.05, N.C. (Next Cycle).

Levels of estradiol 17β(pg/ml) along 17 and 18 days estrous cycle in ewes:

Results showed in table (3) revealed that levels of estradiol 17βshowed fluctuation during 17 days estrous cycle where the highest levels were recorded during 1st2 days of the cycle. By the 3rdday level started to decrease significantly & remained at lowest level during the 7th, 9th, 11th, 13th and 15thdays; after which the level started to increase significantly by the 17thdays then reached the peak by the 1st& 2nddays of the next cycle.

Concerning of the 18 days estrous cycle,data recorded in table (4) indicated that there was fluctuation in estradiol 17β levels during 18 day estrous cycle where the highest values were recorded during 1st2 days of the cycle. By the 3rd day level started to decrease significantly reached the lowest level during the 7th, 9th, 11th, 13th, 15thand 17thdays after which the level started to increase significantly by the 18th day then peaked by the1st& 2nddays of the next cycle.

Concerning the correlation between leptin and gonadal steroids, the data shown in table (7) clearly demonstrated significant positive correlation between leptin levels and progesterone levels and negative correlation between leptin levels and estradiol levels in the 17 days estrous cycle. The data mentioned in the same table reveal that in almost all days of the 18 days estrous cycle,leptin levels were

Levels of progesterone (ng/ml) along 17and 18 days estrous cycle in ewes:

Data presented in table (5) showed that levels of progesterone showed fluctuation during17 days estrous cycle where the lowest level was recorded during 1st2 daysof the cycle. By the 5th day the level stared to increase significantly, reached the highest value by the 11th day and remains so till the15th day. Level started to decline significantly by the 17th day & reached lower values by the 1st& 2nd days of the next cycle.

Concerningof the 18 days estrous cycle,results showed in table (6) revealed that there was fluctuation in progesterone levels during 18 days estrous cycle where the lowest level was recorded during 1st 2 day. Bythe 7th day the level started to increase significantly, reaching the highest value by the 11th day and remained so till 13th day. Level started to declinesignificantlyby the 18th day & reached lower values by the 1st& 2nd days of the next cycle.

significantly positive correlated withprogesterone levels, however leptin levels were negatively correlated with estradiol only at 2nd, 5th, 7th, 13th, 15th, 17th,1st and2nd days of the next cycle, while at the remaining days no significant correlations were recorded between leptin and estradiol.

Table (3): Levels of estradiol 17β (pg/ml) along 17 days estrous cycle in ewes.

	Day1	Day2	Day3	Day5	Day7	Day9	Day11	Day13	Day15	Day17	Day1 of N.C	Day2 of N.C	L.S.D =5.45
Mean	57	56.37	37	16.59	12.62	10.58	11.57	11.35	16.26	43.32	58.73	54.58	
S.E	± 1.2	± 2.36	± 3	± 1.29	± 0.75	± 0.35	± 0.98	± 0.97	± 1.47	± 1.54	± 0.53	± 1.12	

Data indicates mean ± S.E, n = 6, $p < 0.05$, N.C. (Next Cycle).

Table (4): Levels of estradiol 17β(pg/ml) along 18 days estrous cycle in ewes:

	Day1	Day2	Day3	Day5	Day7	Day9	Day11	Day13	Day15	Day17	Day18	Day1 of N.C	Day 2 of N.C	L.S.D =12.92
Mean	57.39	56.03	43.82	21.96	11.35	10.05	8.51	12.74	18.92	40.52	52.45	59.70	51.52	
S.E	±1.12	±5.17	±7.39	± 8.83	± 1.16	± 1.08	± 0.79	± 2.51	± 3.96	± 4.88	± 2.93	± 2.37	± 3.68	

Data indicates mean ± S.E, n = 3, $p < 0.05$, N.C.(Next cycle).

Table (5): Levels of progesterone (ng/ml) along 17 days estrous cycle in ewes:

	Day1	Day2	Day3	Day5	Day7	Day9	Day11	Day13	Day15	Day17	Day 1 of N.C	Day 2 of N.C	L.S.D =0.76
Mean	0.61	0.59	1.1	2.14	3.55	4.52	4.77	4.16	3.05	1.34	0.9	0.55	
S.E	± 0.18	± 0.2	± 0.18	± 0.37	± 0.47	± 0.26	±0.23	± 0.26	± 0.24	±0.22	± 0.1	±0.11	

Data indicates mean ± S.E, n = 6, $p < 0.05$, N.C (Next Cycle).

Table (6): Levels of progesterone (ng/ml) along 18 days estrous cycle in ewes:

	Day1	Day2	Day3	Day5	Day7	Day9	Day11	Day13	Day15	Day17	Day18	Day1 of N.C	Day2 of N.C	L.S.D =1.58
Mean	0.59	0.83	1.06	1.88	3.81	4.35	4.63	3.87	2.57	1.05	0.64	0.47	0.85	
S.E	±0.24	± 0.42	± 0.48	±0.63	± 0.95	± 0.65	± 0.22	± 0.33	± 0.83	± 0.18	± 0.34	± 0.26	± 0.39	

Data indicates mean ± S.E, n = 3, $p < 0.05$, N.C. (Next Cycle).

Table (7): Correlation pattern between estradiol 17β, progesterone and leptin during 17&18 days estrous cycle:

Day	17 days estrous cycle				18 days estrous cycle			
1		P_4	E_2	Leptin		P_4	E_2	Leptin
	P_4	1			P_4	1		
	E_2	-0.834	1		E_2	-0.976	1	
	Leptin	0.957*	-0.802*	1	Leptin	0.958*	-0.874	1
2	P_4	1			P_4	1		
	E_2	-0.895	1		E_2	-0.996	1	
	Leptin	0.956*	-0.792*	1	Leptin	0.999*	-0.995*	1
3	P_4	1			P_4	1		
	E_2	-0.901	1		E_2	-0.928	1	
	Leptin	0.983*	-0.817*	1	Leptin	0.999*	-0.928	1
5	P_4	1			P_4	1		
	E_2	-0.937	1		E_2	-0.999	1	
	Leptin	0.916*	-0.822*	1	Leptin	0.999*	-0.999*	1
7	P_4	1			P_4	1		
	E_2	-0.916	1		E_2	-0.998	1	
	Leptin	0.972*	-0.848*	1	Leptin	0.999*	-0.999*	1
9	P_4	1			P_4	1		
	E_2	-0.940	1		E_2	-0.741	1	
	Leptin	0.811*	-0.953*	1	Leptin	0.995*	-0.806	1
11	P_4	1			P_4	1		
	E_2	-0.992	1		E_2	-0.801	1	
	Leptin	0.969*	-0.981*	1	Leptin	0.991*	-0.716	1
13	P_4	1			P_4	1		
	E_2	-0.982	1		E_2	-0.931		
	Leptin	0.880*	-0.833*	1	Leptin	0.989*	-0.973*	
15	P_4	1			P_4	1		
	E_2	-0.898	1		E_2	-0.977	1	
	Leptin	0.946*	-0.974*	1	Leptin	0.981*	-0.999*	1
17	P_4	1			P_4	1		
	E_2	-0.944	1		E_2	-0.996	1	
	Leptin	0.955*	-0.929*	1	Leptin	0.856	-0.960*	1

Table (7): Correlation pattern between estradiol 17β, progesterone and leptin during 17&18 days estrous cycle(continue):

Day	18 days estrous cycle			
18		P_4	E_2	Leptin
	P_4	1		
	E_2	- 0.863	1	
	Leptin	0.998*	-0.896	1

Day		17 days estrous cycle			18 days estrous cycle			
1st of next cycle		P_4	E_2	Leptin		P_4	E_2	Leptin
	P_4	1			P_4	1		
	E_2	-0.806	1		E_2	-0.981	1	
	Leptin	0.982*	-0.829*	1	Leptin	0.985*	-0.999*	1
2nd of next cycle	P_4	1			P_4	1		
	E_2	-0.913	1		E_2	0.983	1	
	Leptin	0.930*	-0.985*	1	Leptin	0.999*	-0.991*	1

Critical value at 5% of 17 days estrous cycle= 0.755.
Critical value at 5% of 18 days estrous cycle= 0.950.
*Significant correlation.

Discussion

The current study aimed to investigate gonadal steroids and leptin dynamics during the estrus cycle of ewe.The duration of the cycle in ewe is remarkably constant, with some differences in cycle lengths among breeds and with age, however, these differences are relatively small (Hafez, 1952).

The present investigation showed that estradiol levels were highest during the 1st 2 days of the 17 and 18 days estrus cycle, this seemed to be true as estradiol secretion during this period appears to come largely from the follicle destined to ovulate, which is growing rapidly at this time (Bjersing et al., 1972). This result coincided with those of Baird et al. (1981) who reported that estradiol increment peaked at the start of the pre-ovulatory LH surge during estrus phase. Afterwards, the estradiol level starts to decline rapidly and remained at low levels till the 15th day of the cycle. These data agreed with those of Karsch et al.(1979) who showed a rapid fall in estradiol level after its peak to a basal level which remained so as long as the progesterone levels were high. The reported levels showed a significant increase by the 17th and 18th day of the cycle and peaked during the next two days of the subsequent cycle. This result goes hand in hand with that ofBaird et al. (1976) who mentioned a rise in estradiol level concomitant with fall in progesterone level at luteolysis.

Concerning progesterone levels, they behaved reciprocal to those of estradiol and showed a basal level during 1st three days of the 17 and 18 day estrous cycles. This result agreed with that of Hauger et al. (1977) who mentioned that progesterone concentrations were virtually undetectable early in the cycle. The present findings recorded an increase in the progesterone level by the 5th and 7th days of the 17 days and 18 days estrus cycles respectively. This was followed by a peak that persisted through the 9th, 11th and 13th days of the estrus cycle. The present findings agreed in part (not all) with those ofQuirke et al. (1979) who mentioned a gradual rise from days 2 to 8 to reach a maximal concentration that remained relatively constant from days 8 to 14. The discrepancies between the present and the former results may be due difference in sampling time and/or the sheep breed.

While a fall in progesterone level was evidenced by the 15th day of the 17 day estrus cycle, a similar decrease in progesterone level at the 15th day was not recorded in the 18 day estrus cycle. This difference in the fall of progesterone may be the reason behind the difference in cycle length. Rapid and progressive decreases were recorded starting from the day 17th of the estrus cycle and continued through the 1st two days of the subsequent cycle. The rapid decrease coincided with results of Quirke et al. (1979) who demonstrated rapid fall in progesterone by the 15th day of the cycle.

Concerning leptin, a protein hormone, which is mainly secreted by adipose tissue and constituting an important link between metabolism and reproduction where it serves as a metabolic signal informing the reproductive system that sufficient fat stores are available to meet the caloric demands of reproduction. Furthermore, direct control for the ovarian activity is suggested, since in the murine ovary, leptin is expressed in oocytes, granulosa cells, and theca cells where the highest expression of leptin is confined to the oocyte, while the leptin receptor has highest expression intheca cells (Ryan et al., 2002). Levels of leptin show fluctuations along the estrus cycle where lowest levels were recorded during the 1st 3 days and 5 days of the 17 and 18 days estrus cycle respectively. This low level seems to be true during the late follicular phase as leptin can

suppress ovarian estradiol synthesis (Zachow and Magoffin, 1997). This result coincided with those of Spicer and Francisco (1997) and Barkan et al. (1999) who reported that in primary rat granulosa cells, in rat and human granulosa cell lines and in bovine granulosa cells, leptin inhibited aromatase activity, insulin and IGF1 induced production of estradiol. Moreover, passive immunization against leptin during the follicular phase of ewe estrus cycle has resulted in an increased estradiol secretion, while direct exposure of ovaries to physiological doses of leptin at the same stage decreased estradiol secretion (Kendall et al., 2004).These result were further documented by the correlation study in the present investigation which revealed that almost all times estradiol levels were negatively correlated with leptin levels. Thus lower leptin during the estrus phase might have resulted in maximization of estradiol secretion consequently inducing estrus behavior and LH surge. In contrast, the reported result disagreed with those of Kitawaki et al. (1999) who reported that leptin increases aromatase mRNA and protein expression in human granulosa cells. Leptin levels increased by the 5th and the 7th days of the 17 and 18 days estrus cycle respectively. Levels remained elevated till the 13th day and 15thday of the 17 and 18 days estrus cycle respectively, afterwards levels decreased. The elevated leptin levels paralleled those of progesterone and this agreed with previous results showing that leptin infusion resulted in an increase in the steroidogenic capacity of the corpus luteum (Kendall et al., 2004). Additionally, the corpus luteum in pigs (Ruiz-Cortes et al., 2000) and cattle (Nicklin et al., 2004) express leptin receptor, moreover, it has been observed that expression of leptin receptor (Ob-Rb) is highest during di-estrus (Duggal et al., 2002) suggesting stimulatory role on corpus luteum steroidogenesis. These results coincided with those of (Ruiz-Cortes et al., 2000) who showed that leptin binding to its receptors on corpus luteum increases and the increase paralleled to progesterone production. This seemed logic as leptin could aid the development of the corpus luteum in a number of ways: leptin enhances the expression of StAR by luteinizing granulosa cells (Ruiz-Cortes et al. 2003); and could directly increase the blood flow to the corpus luteum (Berisha et al., 2002, Schams et al., 2003).This was further assured by a positive correlation thatexisted between progesterone levels and those of leptin.

Conclusively, leptin levels showed fluctuation along the estrous cycle being low during late follicular phase and increased during luteal phase, moreover the levels were positively correlated with progesterone and negatively correlated with estradiol.

References

Aboul-Naga, A.M.; Aboul-Ela, M.B. and Mansour, H. (1987): Seasonality of breeding activity in subtropicalEgyptian sheep breeds. 38th Annual Meeting EAAP, Lisbon, Portugal.

Baird, D. T.; Land, R. B.; Scaramuzzi, R. J. and Wheeler, A. G. (1976): Endocrine changes associated with luteal regression in the ewe: the secretion of ovarian estradiol, progesterone and androstenedione and uterine prostaglandin F2α throughout the estrous cycle. J. Endocrinol., 69: 275-286.

Baird, D. T.; Swanston, I. A. and McNeilly, A. S. (1981): Relationship between LH, FSH, and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe. Biol. Reprod., 24: 1013-1025.

Barkan, D.; Jia, H.; Dantes, A.; Vardimon, L.; Amsterdam, A. and Rubinstein, M. (1999): Leptin modulates the glucocorticoid- induced ovarian steroidogenesis. Endocrinol., 140: 1731-1738.

Berisha, B.; chams, D. and Miyamoto, A. (2002): The expression of angiotensin and endothelin system members in bovine corpus luteum during estrous cycle and pregnancy. Endocrinol., 19: 305–312.

Bindon, B. M.; Piper, L. R.; Hilliard, M. A. (1996): Reproductive physiology and endocrinology of prolific sheep. In: Fahmy, M.H. (ed.), Prolific Sheep. Wallingford, UK: CAB International; 453-470.

Bjersing, L.; Hay, M. F.; Kann, G.; Moor, R. M.; Naftolin, F.; Scaramuzzi, R. J.; Short, R. V. and Younglai, E. V. (1972): Changes in gonadotropins, ovarian steroids and follicular morphology in sheep at estrus. J. Endocrinol., 52: 465-479.

Doring, H.; Schwarzer, K.; Nuesslein-Hildesheim, B. and Schmidt, I. (1998): Leptin selectively increasesenergyexpenditureof foodrestrictedleanmice. Int.J.Obe. Rel.Metab. Disord., 22: 83–88.

Duggal, P.S.; Weitsman, S.R.; Magoffin, D. A. and Norman, R.J. (2002): Expression of the long (OB-RB) and short (OB-RA) forms of the

leptin receptor throughout the estrous cycle in the mature rat ovary. Reprod., 123: 899–905.

Faber-Nys, C. and Vernier, G. (1987): Development and use of a method for quantifying female sexual behavior in ewes. . Appl. Anim. Behav. Sci., 17: 289-304.

Galal, S.; Abdel-Rasoul, F.; Anous, M.R. andShaat, I.M. (2005): On station Characterization of Small Ruminant Breeds in Egypt. In: Characterization of Small Ruminant Breeds in West Asia and North Africa, 2: 141-193 Luis Iniguez (Ed.) ICARDA,Aleppo, Syria.

Garcia, M.R.; Amstalden, M.; Williams, S.W.; Stanko, R.L.; Morrison, C.D.; Keisler, D.H.; Nizielski, S.E. and Williams, G.L. (2002): Serum leptin and its adipose gene expression during pubertal development, estrous cycle and different seasons in cattle. J. Anim. Sci., 80(8): 2158-2167.

Goodman, R.L. (1994): Neuroendocrine control of the ovine estrous cycle. Phsiol. Reprod., 2nd edition. pp. 660-93.

Hafez, E.S.E. (1952): Studies on the breeding season and reproduction of the ewe. J. Agric. Sci. 42: 189-265.

Hauger, R.L.; Karsch, F. J. and Foster, D. L. (1977): A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinol., 101: 807-817.

Heymsfield, S.B.; Greenberg, A.S.; Fujioka, K.; Dixon, R.M.; Kushner, R.; Hunt, T.;Lubina, J.A.;Patane, J.; Self, B.; Hunt, P. andMcCamish, M.(1999): recombinant leptin for weight loss in obese and lean adults: A Randomized, Controlled, Dose-Escalation trial. JAMA 282(16):1568-75

Hoggard, N.; Hunter, L.; Trayhurn, P.; Williams, L.M. and Mercer, J.G. (1998): Leptin and reproduction. Pro. Nut. Soci., 57(3): 421-427.

Houseknecht, K.L.; Baile, C.A.; Matteri, R.L.; Spurlock, M.E. (1998): The biology of leptin: A review. J. Anim. Sci., 76: 1405–1420

Jin, L.; Burguera, B.G.; Couce, M.E.; Scheithauer, B.W.; Lamsan, J.; Eberhardt, N.L.; Kulig, E. and Lloyd, R.V. (1999): Leptin and Leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. J. Clin.Endocrinol.andMetab., 84(8): 2903-2911.

Karsch, F. J.; Foster, D. L.; Legan, S. J.; Ryan, K. D. and Peter, G. K. (1979): Control of the preovulatory endocrine events in the ewe: interrelationship of estradiol, progesterone, and luteinizing hormone. Endocrinol., 105: 421-426.

Kendall, N. R.; Gutierrez, C. G. ; Scaramuzzi, R. J.; Baird, D. T.; Webb, R. and Campbell, B. K. (2004): Direct in vivo effects of leptin on ovarian steroidogenesis in sheep. Reprod., 128: 757–765

Keyes, p.l.; Gadsby, J.E.; Yuh, K.C. and Bill, C.A. (1983): the corpus luteum. Int. Rev. physiol., 27:57-97

Kitawaki, J.; Kusuki, I.; Koshiba, H.; Tsukamoto, K. and Honjo, H. (1999): Leptin directly stimulates aromatase activity in human luteinized granulosa cells. Mol. Hum. Reprod., 5: 708-713.

Masuzaki, H.; Ogawa, Y.; Sagawa, N.; Hosoda, K.; Matsumoto, T.; Mise, H.; Nishimura, H.; Yoshimasa, Y.; Tanaka, I.; Mori, T. and Nakao, K. (1997): Non adipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat. Med., 3(9): 1029-1033.

Nicklin, L.T.; Glister, C.; Robinson, R.S.; Marsters, P.; Campbell, B.K.; Knight, P.G.; Mann, G.E. and Hunte, M.G.(2004): Leptin in the bovine corpus luteum – receptor expression and effects on progesterone production. Reprod. Abstract Series 31 p 6 Abstract O2.

Quirke, J. F.; Hanrahan, J. P. and Gosling, J. P. (1979): Plasma progesterone levels throughout the estrous cycle and release of LH at estrus in sheep with different ovulation rates. J. Reprod. Fertil., 55: 37-44.

Ractliffe, W.A.; Carter, G.D.; Dowsett, M.; et al., (1988): Estradiol assays: application and guidelines for the provision of a clinical biochemistry service, Ann. Clin. Biochem., 25: 466-488

Robertson, H.A. (1969): The endogenous control of estrus and ovulation in sheep, cattle, and swine. Vitam.Horm., 27:91-130.

Ruiz-Cortes Z.T.; Men, T.; Palin, M.F.; Downey, B.R.; Lacroix, D.A. and Murphy, B.D. (2000): Porcine leptin receptor: molecular structure and expression in the ovary. Molec. Reprod. Develop.,56: 465–474.

Ruiz-Cortes, Z.T.; Martel-Kennes, Y.; Gevry, N.Y.; Downey, B.R.; Palin, M.F. and

Murphy, B.D. (2003): Biphasic effects of leptin in porcine granulosa cells. Biol. Reprod., 68: 789–796.

Ryan, N. K.; Woodhouse, C. M.; Van der Hoek, K. H.; Gilchrist, R. B.; Armstrong, D. T. and Norman, R. J. (2002): Expression of leptin and its receptor in the murine ovary: possible role in the regulation of oocyte maturation. Biol. Reprod., 66:1548-1554.

Scaramuzzi, R.J. and Martin, G.B. (2008): The importance of interactions among nutrition, seasonality and socio-sexual factors in the development of hormone-free methods for controlling fertility. Reprod. Dome. Anim., 43(2): 129-136.

Schams, D.; Berisha, B.; Neuvians, T.; Amselgruber, W. and Kraetzl, W.D. (2003): Real-time changes of the local vasoactive peptide systems (angiotensin, endothelin) in the bovine corpus luteum after induced luteal regression. Mole. Reprod. Develop., 65: 57–66.

Smith-Kirwin, S.M.; O'Conor, D.M.; De Johston, J.; Lancey, E.D.; Hassink, S.G. and Funanage, V.L. (1998): Leptin expression in human mammary epithelial cells and breast milk. J. Clin. Endocrinol.Metab., 83(5): 1810-1813.

Snedecor, G.W. and Cochran, W.G. (1980): statistical methods.7th edition. Iowa state university press. Ames

Sobhani, I.; Vissuzaine, C.; Buyse, M.; Kermorgant, S.; Laigneau, J.P.; Attoub, S.; Lahy, T.; Henin, D.; Mignon, M. and Lewin, M.J. (2000): Leptin secretion and leptin receptor in the human stomach. Gut, 47 (2) : 178-183.

Spicer, L.J. and Francisco, C.C. (1997): The adipose obese gene product,leptin: evidence of a direct inhibitory role in ovarian function. Endocrinol., 138: 3374–3379.

Tietz, N.W. ed. (1995): Clinical Guide to laboratory Tests, 3rd Edition, Saunders, W.B., Co., Philadelphia, 216-217.

Wang, J. (1998): A Nutrient sensing pathway regulates leptin gene expression in muscle and fat. Nat., 393(6686): 684-688.

Zachow, R. J.and Magoffin, D.A. A. (1997): Direct intraovarian effects of leptin: impairment of the synergistic action of insulin-like growth factor- I on follicle-stimulating hormone-dependent oestradiol-17beta production by rat ovarian granulosa . cells. Endocrinol., 138:847–850.

Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.and Friedman, J.(1994): Positional cloning of the mouse obese gene and its human homologue. Nat., 372:425–32.

الملخص العربى

هرمون اللبتين يعتبر إشارة لمستوى الطاقة فى الجسم التى تؤثر بدورها على الكفاءة التناسلية. بحثت الدراسة الحالية فى ديناميكية هرمونات المناسل الاستيرودية واللبتين أثناء دورة الشبق فى النعاج : دراسة ارتباطية وتم استخدام تسعة نعاج فى هذه الدراسة بعد إحداث تزامن فى دورة الشبق وتم أخذ عينات الدماء أثناء دورة الشبق وأوضحت الدراسة إلى تموج فى مستوى اللبتين أثناء دورة الشبق حيث كان فى أعلى مستوى له فى الفترة التى تلت التبويض بينما كان فى مستوى منخفض فى الفترة ما قبل التبويض.بالنسبة لهرمون الاستراديول فإنه سجل أعلى مستوى له أثناء الأيام الأولى من دورة الشبق وكذلك فى الأيام الأخيرة بينما كانت مستوياته فى الحدود الدنيا خلال الفترة ما بعد التبويض إلى اليوم الخامس عشر من الدورة بينما سجل هرمون البروجسترون أقل مستوى له أثناء الأيام الأولى من دورة الشبق وسجل أعلى مستوى له فى اليوم الحادى عشر من دورة الشبق.والخلاصة فإن مستويات هرمون اللبتين تختلف أثناء دورة الشبق وترتبط إيجابا مع هرمون البروجسترون وسلبا مع هرمون الاستراديول.