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Effect of Age and Physiological Status on Some Serum Energy Metabolites and Progesterone in Ouled Djellal Breed Ewes in Algeria

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***Abstract*—**The aim of this study is to determine the effect of age and physiological status on progesterone and energy metabolism of Ouled Djellal (O.D) breed ewes. 40 healthy ewes were divided into two groups, primiparous and multiparous, with 20 ewes in each group. The body weights (BW) (Kg) were 46.6 ± 4.20 and 59.2 ± 3.02, and consuming less 25 to 30% of their basal energetic requirements. The values of serum glucose, triglycerides and cholesterol were lower in pregnant than in non-pregnant ewes. The high to very high significant differences were found during the 15th week of pregnancy for glycaemia and triglyceridemia respectively. Concerning serum progesterone, a very highly significant difference ($p<0.001$) was noted in the pregnant group, and the values were higher in MP than in PP. After lambing, the triglyceridemia values were slightly lower in primiparous than in multiparous pregnant ewes. In order to prevent imbalance during critical periods of reproduction, we can use the serum metabolic profile.

***Keywords*—**Age, Energy metabolites, Ouled Djellal breed ewes, Physiologic status, Progesterone.

I. Introduction

IN North African countries especially in the steppe that is characterized by a semiarid in the northern and arid climates in the southern fringes; sheep mate in spring and lamb during late summer and autumn. These periods are characterized by extremely hot and dry conditions and scarce pasture vegetation [1], [2]. In Algeria, sheep raising is concentrated especially in the steppe. The O.D breed is the most dominant in this region representing nearly 60% of the 22.8 million heads [3].

Many studies had established some important relationships between nutrition and reproduction. The benefits of dietary nutrition on reproduction in sheep have been described through its influence on embryonic and early foetal development; the size, vigour and viability of the newborn and adult ovulation [4]. Changes in live weight induce changes in ovarian function and ovulation rate influencing therefore the reproductive parameters. Live weight is correlated with body condition; thus, body condition scoring "BCS" is also a suitable parameter to estimate adjustment in animal requirements to available feeds in a practical and simple ways [5]. BCS has a significant effect on metabolites and hormonal profiles. In the same way, the ewes succeed to surmount the extreme nutritional conditions. Although a BCS of 3.0 appear to be ideal to insure nutritional and metabolic welfare; however, with a BCS below 2.0 or above 3.0, the ewes appear exposed to metabolic imbalances [6].

Glucose as a source of energy is necessary for production and reproduction performances [7]. According to individual status, the difference in serum glucose and cholesterol is registered with pregnancy progress [8].

Therefore, during pregnancy, maternal tissues participate to providing energy for reproduction processes, affecting blood serum chemistry values, also linked with several other factors such as breed, age, malnutrition, foetal growth, or season [9].

The nutrition and progesterone represent factors influencing the success of embryonic survival during the early conception [10], [11]. The serum progesterone assay is one of the other diagnostic methods of pregnancy [12]. In a pregnancy diagnosis 16 -20 days after mating, higher progesteronemia levels is an indication of multiple lambing [13].

The aim of this study was to determine the influence of age and physiological status and level of dietary nutrition on progesterone and metabolic parameters during pre-pregnancy, pregnancy and post pregnancy (early lactation) in primiparous and multiparous O.D ewes.

II. Material and Methods

The study was conducted from March 2007 to October 2007.

A. Animals

A total of 40 clinically healthy O.D ewes were used in this study. They were divided into 2 groups, one of 20 primiparous ewes aged than less 2 years (13 pregnant (PP) [10 carrying 1 fetus and 3 carrying 2 fetuses] and 7 non-pregnant (PnP)]. The other one of 20 multiparous ewes (13 pregnants (MP) [7 carrying 1 fetus and 6 carrying 2 fetus] and 7 non pregnant (MnP)] with more than 2 pregnancies and aged between 3 and 6 years. The mean BWs (Kg) were 46.6 ± 4.20 and 59.2 ± 3.02 for primiparous and multiparous respectively. The value of body condition is based on the scale given by Russel et al. [5].

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Synchronization of estrus was taken by the ram effect, where the rams were separated for two months prior to mating.

The study was carried out at ITELV- Ain M'lila (Institut Technique d'Elevage) a semiarid in north steppic region of eastern-Algeria.

B. Serum Assay Procedures

240 blood samples were collected from 40 ewes into Vacuum Venoject® tubes from jugular vein during spring sexual season. The serum parameters were evaluated on different periods: prior to mating (PM), and during early pregnancy (EP), 10 weeks of pregnancy (10w), 15 weeks (15w), late pregnancy (LP) and during early lactation (EL).

5 ml of serums were collected after separation and centrifugation at 3000 x for 15min.

The serum was stored at -20°C until assayed for glucose, cholesterol, triglycerides and progesterone. Glucose, cholesterol and triglycerides concentration was estimated by enzymatic colorimetric test, using Cobas® reagents in biochemical auto analyzer HITACHI-ROCHE 912. Progesterone was analyzed by radioimmunoassay methods in Multi-Crystal Gamma Counter- Berthold LB 2103 using the Coat-A-Count ® Progesterone procedure.

The statistical analysis of data was performed using SYSTAT 12 software 2007. To compare overall parameters studied, two-way repeated measures analysis of variance (ANOVA) was used for comparison of pregnant and non-pregnant ewes to determine the effect of two factors e.g. age and periods of blood sampling. Student test-t was used for measuring the blood parameters and establishes a comparison between groups. Pearson correlation test was applied to establish relationships between the parameters under study and between groups.

C. Feeding Schedule

It is realized by grazing on fallow and stubble in the spring and summer with distribution of barley straw and a concentrate ONAB (Office National des Aliments de Bétail) during spring. During the flushing and early pregnancy, ewes receive the concentrate at a level of 250g/ewe/day. The concentrate is not distributed when the animals graze stubble at least during the first days after harvest. In late pregnancy and early lactation, ewes receive barley straw, oat vetch hay and ONAB concentrate (at a level of 400 g/ewe/day). The concentrate is composed of corn (80 %), byproduct seeds (12%), soybean meal (TS44) (5%), vitamin-mineral premix (2 %) and salt (1%). Stones to lick are at the disposal of the animals of the exploitation.

III. Results

A. Feeding Schedule; Diet Composition and Recommendation

The feed intake and recommended intake are summarized in Table I. The feeding schedule that subordinated during the study in the farm reveals difference between the diet consumed by ewes and that recommended for intake. However, this difference is not important during the different stages of pregnancy except in pre-mating period, late pregnancy and early lactation where energy and protein is reduced by roughly 20-30% than diet recommendation. Concerning the minerals, no important decrease in diet consumed than that recommended by INRA [14]. In addition, during the 2nd and 3rd months of pregnancy, period coinciding with grazing on straw, the consumed food is largely sufficient to cover the needs of the ewes.

B. Levels of Energetic Serum Metabolites

The values of glycaemia (see Table II) are lower in pregnant than in non-pregnant ewes and lower than reference literature ones, with respectively 2.66±0.78, 266±0.72, 2.89±0.72 and 2.89±0.50 mmol/l. The lowest values (in MP: 1.94±0.89 and PP: 2.00±0.83 mmol/l) are noted during the second half of gestation (15w.), coinciding thus with the end of grazing on stubbles and crops. A highly significant difference was noted between the groups of multiparous ($p<0.01$) and significant difference between primiparous groups ($p<0.05$). The analysis of Pearson correlation did not reveal any important relationship between glycaemia and the other metabolites. There was a significant interaction between sampling time and age ($p<0.05$).

The data of serum triglycerides (Table II) are globally in accordance with the literature values. In short, the lowest values are noted in pregnant than in non-pregnant ewes and the highest are observed in the primiparous non-pregnant ewes. At a 15w. of pregnancy a very high significance in non-pregnant group (MnP vs PnP) with $p<0.001$ and high significance was observed with $p<0.01$ in primiparous group (PP vs PnP). The analysis of Pearson correlation did not show any significant relationship between metabolites (Table III); whereas, the ANOVA had revealed an effect certain of time sampling with $p<0.01$ ($p=0.006$) and the same observation is noted in the pregnant group with $p<0.01$($p=0.002$). Besides, the important gaps were noted in the data of the pregnant groups.

The levels of serum cholesterol (Table II) in all groups are in the ranges of the literature values. The mean values were low in pregnant group than in non-pregnant group with 1.42 ±0.31, 1.37±0.28, 1.63±0.26 and 1.55±0.36 respectively in MP, PP, MnP and PnP. Significant differences ($p<0.05$) were found during early pregnancy in pregnant ewes and in primiparous groups and at periods 10w.; and during LP in multiparous group. The ANOVA analysis for data set shows a high significant effect of age with $p<0.001$.

TABLE I
ANALYTICAL FEED INTAKE COMPOSITION AND RECOMMENDED INTAKE

Periods	Premating (PM) and early pregnancy (EP) Concentrate (250g/ewe/day)				Late pregnancy (LP) and early lactation (EL) Concentrate (400 g/ewe/day)			
	Primiparous ($P^{0.75}$= 17.66)		Multiparous ($P^{0.75}$=21.15)		Primiparous		Multiparous	
Age	*D.C*	*R.I.*	*D.C*	*R.I.*	*D.C*	*R.I.*	*D.C*	*R.I.*
VDMI	0.79	0.79	0.89	0.89	1.02	1.02	1.12	1.12
UF	0.65	0.93	0.75	1.02	0.88	1.20	0.98	1.30
PDI (g)		53		62		112		132
PDIN	38		41		64		68	
PDIE	57		63.5		83		89	
Rmic.		-19		-22.5		-19		-21
Ca (g/day)	2.0	3.9	2.47	4.5	3.4	10.3	3.9	11.8
P (g/day)	1.7	2.5	1.83	3.0	3.77	4.4	4.1	4.1

D.C= diet concentration, PDI= protein truly Digestible in the small intestine, PDIE = true protein absorbable in the small intestine when rumen fermentable energy (organic matter) is limiting microbial protein synthesis in the rumen, PDIN = true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen, R.I= recommended intake [14], Rmic= equilibrium between PDIN-PDIE from ration (= PDIN-PDIE/UF),UF = Feed Unit ,VMDI = Voluntary dry matter intake.

C. Progesterone Profile

The serum progesterone (Table IV) showed a gradual increase of the progesterone with the advancement of the gestation with the most elevated values at the end of gestation and their decrease after parturition to reach the base levels. The multiparous ewes presented more elevated values than primiparous ewes during all periods. There is evidence during gestation in which we noted a very highly significant difference ($p< 0.001$) between pregnant and non-pregnant ewes due to their physiological status.

TABLE II
MEAN CONCENTRATION (±*SD)* OF SOME ENERGETIC BLOOD METABOLITES IN EWES DURING DIFFERENT PERIODS

Glycemia (mmol/l)		PM	EP	10w	15w.	LP	EL
	MP	2.54 ±0.82	2.48±0.68	2.92±0.72	1.92±0.87^{c**}	2.89±0.48	3.11±0.29
	PP	2.54±0.78	2.65±0.89	2.99±0.45	2.00±0.81^{d*}	2.91±0.45	2.92± 0.46
	MnP	2.64±0.79	2.75±0.36	3.20±0.49	2.85±0.29	2.74±0.34	3.03±0.46
	PnP	2.62±0.28	2.34±0.68	3.28±0.82	2.94±0.37	2.78±0.28	2.95±0.26
References values		2.78- 4.44 [7],[15] ; 2.3-4.2 [16]					
Triglycerides (mmol/l)	MP	0.38 ±0.17	0.25±0.07	0.38±0.28	0.27 ±0.21	0.32±0.17	0.27±0.13
	PP	0.28±0.11	0.28±0.11	0.45±0.22	0.23±0.11 ^{d**}	0.45±0.26	0.27±0.06
	MnP	0.44 ±0.26	0.30±0.10	0.36±0.18	0.23±0.07	0.37±0.12	0.27±0.08
	PnP	0.40 ±0.26	0.41±0.17	0.42±0.21	0.57±0.17 ^{b***}	0.35±0.10	0.30 ±0.07
References values		1.05-1.50 [7] ; 0.57± 0.21 [16] ; 0.14-0.44 [17]					
Cholestrol (mmol/l)	MP	1.53±0.26	1.50±0.28 ^{a*}	1.30±0.28 ^{c*}	1.35±0.47	1.42±0.28 ^{c*}	1.40±0.31
	PP	1.45±0.26	1.24±0.26 ^{d*}	1.48±0.21	1.30±0.31	1.42±0.26	1.40±0.31
	MnP	1.58±0.23	1.63±0.16	1.55±0.23	1.81±0.34	1.63±0.18	1.55±0.28
	PnP	1.45± 0.39	1.76±0.52	1.55±0.31	1.53±0.31	1.42±0.28	1.55±0.31
References values		1.35 – 1.97 [15] ; 1.34-1.96 [16]					

^{a}= MP vs PP, ^{b}=MnP vs PnP, ^{c}= MP vs MnP, ^{d}= PP vs PnP, *= p<0.05, **= p< 0.01, ***= p<0.00, w.= week.

TABLE III
PEARSON CORRELATION MATRIX

	Glucose	Triglycerides	Cholesterol	Progesterone
Glucose	1.000			
Triglycerides	0.136	1.000		
Cholesterol	0.259	0.216	1.000	
Progesterone	-0.207	-0.013	-0.169	1.000

TABLE IV
MEAN CONCENTRATION (±*SD)* OF PROGESTERONEMIA IN EWES DURING DIFFERENT PERIODS

progesterone (ng/ml)		PM	EP	10w	15w.	LP	EL
	MP	1.40±0.61	4.47±1.13	8.54±2.73 ^{a**}	17.43±5.26 ^{a*}	20.67±4.01	0.09±0.46
	PP	1.24±1.68	3.56±1.10^{d***}	5.27±1.83 ^{d***}	11.46±5.49 ^{d***}	18.95±5.28 ^{d***}	0.10±0.55
	MnP	1.00±0.94	0.94±1.08 ^{c***}	1.11±1.24 ^{c***}	3.13±0.59 ^{c***}	3.01±1.06 ^{c***}	1.50±1.18 ^{c*}
	PnP	1.05±1.10	0.20±0.35	0.43±0.78	1.95±027^{b***}	2.63±1.13	1.10±1.17
References values		2.77±0.31 and 2.31± 0.31(at 17 and 35 days)(in O.D ewes [18]). 1.41±0.21 (0-6d) ; 4.0±0.87 (16-30d) ; 4.6±1.08 (60-75d) ; 10.81±2.10.9±1.1 (17th w); 8.5±0.8 (20th w) 5.2 ±0.9 (21th w); 0.1±0.2 (1st w. after lambing) (in O.D ewes [19]) 85(91-105d) ;13.33±2.43 (136- lambing); 0.26±0.03 (8-15d post-lambing) (in Corriedale ewes [12]).					

^{a}= MP vs PP, ^{b}=MnP vs PnP, ^{c}= MP vs MnP, ^{d}= PP vs PnP, *= p<0.05, **= p< 0.01, ***= p<0.00, d= day, w= week.

During the mid-gestation (10 and 15 weeks) a significant difference between MP and PP ewes ($p<0.01$ and $p<0.05$ respectively was noted. However, at the 15th week, a very highly significant difference was revealed between the ewes in the non-pregnant group with $p<0.001$ resulting probably from the ovarian activity especially in MnP than in PnP with respectively 3.13 ± 0.59 and 1.95±0.27 ng/ml. This ovarian activity continued in this group without installation of pregnant state among some ewes thereafter, according to the decreased values in the sixth sampling time (EL) with 1.50±1.18 and 1.1±1.17 ng/ml in MnP and PnP respectively. The very weak negative relationship exists between serum progesterone and glucose (r= -0.207) and between progesterone and cholesterol (r= - 0.169) (see Table III). The ANOVA shows a very high significant effect of age, sampling time and age x sampling time ($p<0.001$).

IV. Discussion

The energy requirements vary in animals with different factors such as age, sex, live weight, body condition, physiological status, environmental conditions, physical activity and genetic characteristics [6]. When the animals' net requirements are more than the net nutrient intake especially on energy, they will utilize their energy stores to meet the lack. In this state, the animal is in "negative energy balance" [20]. The animals adapted to low digestible energy on available diet can modify significantly those energy requirements [21].

Concerning the ration distributed to ewes, we noted that the supplement to the basic ration (250g of concentrate/ewe/day) during this phase of preparation is weak comparatively to the recommendations for the flushing.

The values of glycaemia are higher than those found by [22] and lower to those reported by [23], [24]. During late pregnancy and early lactation, the values of glycaemia are roughly similar to those obtained by [25] in Barbarine breed ewes receiving a diet supplemented with barley. On the other hand, Dell'Orto et al. [26] did not observe any difference between ewes receiving a concentrate in diet with high starch concentration (34.1%) and those receiving lower one (12.2%).

According to Ehrhardt et al. [23] and Robinson [27], the energy requirements had an important effect especially on reproduction. This effect is more important in females than in males because of the demand of gestational development and milk production. The utilization of glucose by utero-placental and foetal tissues may account for at least 35% of maternal glucose production during the last few weeks of pregnancy in sheep [28]. According to Fisher et al. (1974) cited by [29] many factors could affect concentration of blood metabolites in ruminants. However, blood glucose concentration could be used to predict energy intake and efficiency of utilization. In addition, the reduction of energy induces some pathology such as in late pregnancy the toxemia with hypoglycemia, hyperketonaemia and increase in concentration of free fatty acids with fatty infiltration of the liver [30], [31]. Low value of glycaemia is significantly correlated with reduced glucose concentration in the cerebrospinal fluid [32].

According to some authors, the ruminant plasma concentration of triglycerides is very low than in other species and the triglycerides secretion is limited during energy deficiency and increased lipomobilisation [31]. Also, during the mid-term undernutrition, the homeostatic regulation of the lipogenic pathways is probably exacerbated by short-term changes in plasma concentrations of insulin and lipogenic substrates particularly the rise in NEFA, and the decrease in acetate and triglycerides [33], [34]. Also, during pregnancy a decrease in lipoprotein lipase activity has been observed leading thus to a hyperglyceridemia. It entails a reduction and an alteration of the secretion and the catabolism of the triglycerides [31]. According to Caldeira and Portugal [33], there is a high correlation between BC than live weight with fat serum parameters particularly triglycerides and free fatty acids. The values of serum triglycerides are globally higher than those obtained by [33] and similar to pregnant ewes feed-restricted (0.21± 0.01 mmol/l) and lower to pregnant ewes on adequate diet in a study by [31]. Our values are lower than those obtained by [35] in all groups (control, sublinic and clinic toxemia) pregnant Akkaraman ewes and to those showed by [29] in ewes subjected to different dietary energy and phosphorus. During pregnancy and lactation, the values of triglyceridemia are lower than those obtained by [22].

Concerning the serum cholesterol, we note a decrease in values mainly in pregnant ewes. These values are in agreement with those reported by [36], especially during the late gestation and early lactation.

The lowest values are noted in primiparous pregnant ewes during early pregnancy and the 15th week with respectively 1.24±0.26 and 1.30±0.31 mmol/l, these values are lower compared to those reported by [16] (1.34-1.96 mmol/l). In all groups, the values are lower than those described by [29] with diets containing different rates of energy and phosphorus, and then those showed by [35]. In general the values of cholesterol are lower than those obtained by [37], [38] during different stages of pregnancy and then those observed by [39], [40]. However, we noted an elevation of the cholesterolemia with the stage of advancement of the gestation, what is in agreement with the observations of [37], [38]. During lactation, both groups of lactating ewes present lower values than those observed by [39], [41]. The slightly higher level of value in late gestation than in lactation period is due to the mobilization of liver lipoproteins during pregnancy [39]. Therefore, the decrease during lactation could be due to the raised cholesterol uptakes by tissues involved in milk synthesis because of normal insulin responsiveness compared to late pregnancy [8], [36], [42].

The values of progesterone in pregnant group are higher than those obtained in O.D ewes by [19] and in Corriedale ewes by [12], while following the same tendency to the increase with the progression of the gestation. Equally, we note an equivalence to those obtained by [13], [38] in the second mid-gestation, but lower to those obtained by the last authors at 120th day with 30±4.9 ng/ml. The levels of progesterone in data (means during gestation in MP: 11.67±7.68 and PP : 9.07±6.78 ng/ml) are lower than those

obtained by [43] who reported 13.2±1.0 and 18.7±1.0 ng/ml in Javanese Thin-Tail pregnant ewes with 1 and 2 fetus respectively. In the same order, [19] noted a difference in values of progesteronemia between ewes with single or multiple fetuses, values that are elevated in this last group.

According to Ganaie et al. [12] and Tamassia [44] the level of progesterone with respectively ≥ 1.75 and ≥2.5 ng/ml is considered as a sign of pregnancy that remains elevated beyond day 18 post-mating in pregnant ewes, while it decreases in ewes that beached to conceive. The high level of serum progesterone over the luteal phase of the estrous cycle after mating is indicative to high conception rates [45] and there was a strong relationship between progesterone production and lamb birth-weight [43], [46]. The reduction in pregnancy rates of ewes with peripheral progesterone concentrations above 5 ng/mL on day 12 was probably due to direct effects of undernutrition rather to elevated progesterone. In other hand, the increase in serum concentrations of progesterone is noted in response to feed restriction in sheep [10], [47]. Also, feed withdrawal during luteal phase of the estrous cycle increases serum concentrations of progesterone and evoked endocrine changes that could perturb the subsequent estrous cycle [48].

V. Conclusion

The present study indicated that metabolic and hormonal profiles are influenced by age and physiological status in association with the level of nutrient feeding. The great variations in biochemical metabolites are observed during the second mid-pregnancy coinciding with the end of grazing in stubbles and the scarce of feed. The level of progesterone in blood is maintained continuously elevated during pregnancy and decreases thereafter in early lactation. Also, the ewes that receive a lower level of nutrient during the pregnancy present some metabolic imbalance especially, and there is imperative need to introduce a balanced ration which ensures a supply of essential nutrients in a proper ratio during all physiological status.

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