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Influence of Age and Physiological Status on Progesterone and Some Blood Metabolites of Ouled Djellal Breed Ewes in East Algeria

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Abstract: The aim of this study is to determine the influence of age and physiological status on progesterone and metabolic profile of Ouled Djellal (O.D) breed ewes. Investigations were carried out on 40 healthy O.D ewes that were divided into 20, two year old primiparous ewes (13 pregnant (PP) and 7 non-pregnant (Pn-P)) and 20 multiparous (13 pregnant (MP) and 7 non pregnant (Mn-P)) ewes with more than two pregnancies aged between 3 and 6 years. The BW (Kg) were 46.6 ± 4.20 and 59.2 ± 3.02 for PP and MP respectively and consuming less 20% of their basal requirements. Six serum sampling sets were realized during different periods corresponding to one cycle of reproduction. The values of serum total proteins, albumin and urea were higher in non pregnant than in pregnant ewes. A very highly significant difference ($p<0.001$) was noted in pregnant group, concerning serum progesterone a significant effect of age, sampling time and age x sampling time is traced. In all protein metabolites studied ($p<0.05$) in non-pregnant group and total protein and albumin ($p<0.01$) in pregnant group, the effect of sampling time represent the major factor influencing the variation between groups. After lambing, the values compared to pregnancy values were lower for proteinic metabolites and they were lower in PP than in MP ewes. The progesterone values were higher in MP than in PP. However, the values of metabolic profile during 15w. period were lower than the norms. The results obtained in this study recommend to justify the use of the serum metabolic profile, which varied with age and physiological status, in order to cover their requirements according to physiological status and to prevent imbalance during those critical periods.

Key words: Ouled Djellal Ewes · Age · Pregnancy · Blood Metabolites And Progesterone

INTRODUCTION

In Algeria, sheep raising is concentrated in the steppe and the mutton is the most favorable meat. The Ouled Djellal (OD) breed is the most dominant in this region representing nearly 60% of the 19.6 millions heads [1]. The climate of the steppe is characterized by cold winters and hot dry summers. The steppe ecosystem is characterized by a great variability of rain precipitations. Therefore; the major constraints to raise productivity in this poor climatic conditions represents a major concern of sheep breeders [2].

Most of the studies had established several important relationships between nutrition and reproduction. The benefits of nutrition on reproduction in sheep have been described through its influence on embryonic development; the size, vigour and viability of the newborn and ovulation [3]. The challenge of adequate feeding during late pregnancy and early lactation in ewes is to sustain the accelerated growth of the foetus [4]. Therefore, the production of viable lambs requires that ewes should be in good health during and after pregnancy. Metabolic profiles are used to diagnose metabolic diseases and to assess the nutritional status to predict the metabolic problems in peripartum periods [5].

The nutrition and progesterone represent factors influencing the success of embryonic survival during the early conception [6, 7]. The use of serum progesterone is one among other diagnosis methods of pregnancy at an early stage reducing also the economic impact in sheep production [8].

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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.

MATERIALS AND METHODS

The present study was conducted from March to October 2007.

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 of less 2 years (13 pregnants (PP) [10 carrying 1 fetus and 3 carrying 2 fetuses] and 7 non-pregnants (Pn-P)) and the other one of 20 multiparous ewes (13 pregnants (MP) [7 carrying 1 fetus and 6 carrying 2 fetus] and 7 non pregnant (Mn-P)) with more than 2 pregnancies and aged between 3 and 6 years. The mean BW (Kg) were 46.6 ± 4.20 and 59.2 ± 3.02 for primiparous and multiparous respectively. The evaluation of body condition is based on the scale given by Russel A. [9].

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

The study was performed on a farm belonging to the ITELV- Ain M'lila (Institut Technique d'élevage) located south of the city in steppic region of eastern-Algeria with an average annual rainfall of 250- 350 mm.

Serum Sampling and Assay Procedures: 240 blood samples were collected in vacuum Venoject® tubes from jugular vein during breeding season (spring). The studied parameters were evaluated for each ewe on different periods: prior to mating (PM), early pregnancy (EP), 10 week of pregnancy (10 w.), 15 week (15 w.), late pregnancy (LP) and during early lactation (EL).

The collected serum was stored at -20 until assayed for total proteins, albumin, urea and progesterone. Total proteins were estimated by colorimetric method (Biuret reaction), albumin by Jaffe method and urea by Talk and Schuber method, using Cobas® reagents in biochemical Auto Analyzer HITACHI-ROCHE 912. Progesterone was analyzed by a radio-immunoassay method 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. t- test was used for comparison between groups. Pearson correlation test was applied to study relationships between the parameters under study and between groups.

Feeding Schedule: It is realized by grazing on fallow and stubble during 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 received 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 bran (12%), soybean meal (TS44) (5%), vitamin-mineral premix (2 %) and salt (1%). Stones to lick are at the disposal of the animals.

RESULTS

The feed intake is summarized in Table 1. The feeding schedule during the study revealed a 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 are reduced by roughly 20- 30 % than the recommended diet. Concerning the minerals, there was no important reduction in reference to that recommended by INRA [10]. On the other hand, during the 2nd and 3rd months of pregnancy, the consumed food is largely sufficient to cover the needs of the ewes.

The metabolic profile of total proteins is summarized in Table 2 and the results showed no difference between groups of age or physiological status, but the only significant differences ($p<0.05$) are observed at 10w (MP vs PP) and at 15w. (Mn-P vs Pn-P and PP vs Pn-P). A significant effect of sampling time ($p <0.01$) and ($p <0.0005$) has been observed on non-pregnant ewes and pregnant ewes respectively, but the effect of age is significant only in pregnant ewes. Also, for all groups, there was clear effect of time of sampling ($p <0.0005$) more than the effect of age ($p <0.05$).

Table 1: Analytical feed intake composition and recommended intake

Periods	Premating and early gestation Concentrate (250g/ewe/day)				Late gestation and early lactation 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		92		101	
PDIE	57		63.5		100		108	
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	4.9

- D.C= diet concentration - R.I= recommended intake (INRA, 1988)

-VMDI = Voluntary dry matter intake

-UF = Feed Unit -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

Table 2: Mean concentration (±*SD)* of some nitrogen blood metabolites in ewes during different periods

Toatl protein (g/L)		PM	EP	10w.	15w.	LP	EL
	MP	74.35±6.630	65.43±9.35	78.22±7.50^{a*}	66.29±14.87^{b*}	77.69±14.47	65.91±9.30
	PP	68.01±9.630	63.90±10.45	72.07±5.06	64.88±9.02d*	77.49±12.06	64.94±9.28
	Mn-P	73.93±12.18	66.86±8.53	71.61±6.53	69.56±5.16	70.20±1.64	69.17±6.98
	Pn-P	73.76±7.950	70.06 ±6.89	78.85±8.20	72.93±4.28	71.23±6.54	65.76±6.34
References values	60-79 [13]; 72 ±5,2 [12]; 67.17±14.02 [22]						
Albumin (g/L)	MP	31.37±3.47^{a*}	27.55±2.90	30.36±3.84	28.10±5.54	32.27±5.67	32.68±9.72
	PP	28.38±3.98^{d*}	27.87±3.69d*	30.25±3.92	28.94±4.56	32.64±6.55	29.49±3.82
	Mn-P	30.82±2.94	28.80±1.94	31.11±4.50	30.47±1.60	30.47±2.77	30.60±1.64
	Pn-P	31.33±2.07	30.81±2.13	31.18±2.45	29.00±2.15	29.57±3.50	28.93±1.32
References values	24-30 [13]; 25-35 [12, 21]; 24.55±8.47 [22]						
Urea (mmol/L)	MP	4.50±1.83	5.33±1.50	4.00±1.00^{a*}	5.00±1.33	3.66±1.17	4.50±1.50
	PP	4.16±1.50	6.49±2.16	5.33±2.00	5.66±1.67	3.66±1.00	4.00±1.50
	Mn-P	3.83±1.50	5.33±2.50	4.33±1.67	5.66±1.50	4.00±0.50	5.49±2.16
	Pn-P	4.5±0.830	5.83±1.67	5.16±1.33	5.00±1.17	4.00±1.00	4.33±2.00
References values	3-8 [21]; 2.86-7.14 [13]; 4.6±2.0 [22]						

-^{a}= MP vs PP; ^{b}=Mn-P vs Pn-P; ^{c}= MP vs Mn-P and ^{d}= PP vs Pn-P. -*: p<0.05; **: p< 0.01; ***: p<0.00. –w.: week

Table 3: Pearson correlation matrix

	Total Proteins	Albumin	Urea	Progesterone
Total proteins	1.000			
Albumin	0.675	1.000		
Urea	-0.152	-0.154	1.000	
Progesterone	0.131	0.036	-0.099	1.000

Table 4: Mean concentration (±SD) of progesteronemia in ewes during different periods

Progsterone (ng/mL)	PM	EP	10w.	15w.	LP	EL
MP	1.40±0.61	4.4.7±1.13	8.8.54±2.73^{a**}	17.43±5.26^{a*}	20.67±4.01	0.09±0.46
PP	1.24±1.68	3.3.56±1.10^{d***}	5.5.27±1.83^{d***}	11.46±5.49^{d***}	18.95±5.28^{d***}	0.10±0.55
Mn-P	1.00±0.94	0.0.94±1.08^{c***}	1.1.11±1.24^{c***}	3.13±0.59^{c***}	3.01±1.06^{c***}	1.50±1.18^{c*}
Pn-P	1.05±1.10	0.0.20±0.35	0.0.43±0.78	1.95±027^{b***}	2.63±1.13	1.10±1.17

-^{a}= MP vs PP; ^{b}=Mn-P vs Pn-P; ^{c}= MP vs Mn-P and ^{d}= PP vs Pn-P. -*: p<0.05; **: p< 0.01; ***: p<0.001. - ^{w}: week

The values of serum albumin (Table 2) were higher in pregnant than in non-pregnant ewes during the late pregnancy and early lactation periods and lower during the first four sampling times, with no significant difference between groups. This parameter followed the same trend as the total proteins. Moreover, a very important correlation between these two parameters (r=0.675) (Table 3) has been found. Serum albumin showed a significant difference ($p < 0.05$) between *MP vs PP* during the pre-mating period. The comparison between primiparous ewes (*PP vs Pn-P)* revealed the same difference during pre-mating and early pregnancy periods.

Serum urea values (Table 2) ranged within the physiological norms cited in the literature. The concentrations in all groups were respectively 4.50±1.50, 4.83±2.00, 4.83±1.83 and 4.83±1.50 (g/l) for MP, PP, Mn-P and Pn-P. If we take solely in consideration, the 4 samples during pregnancy, we note that more elevated values were in primiparous more than multiparous ewes; and the only difference is noted at 10w. time sampling ($p < 0.05$) in pregnant groups. Some effect of age and time sampling (e.g. physiological stage) ($p < 0.02$) of each of the two factors taken separately, but the interaction of the two associated factors did not show a significance (p=0.15). There were no significant negative correlations between urea, total proteins and albumin.

The serum progesterone (Table 4), showed a gradual increase with the advancement of the gestation with the most elevated values at the end of gestation and its decrease after parturition to reach the base levels. Multiparous ewes presented higher values than primiparous ones during all periods.

A very highly significant difference ($p < 0.001$) has been noted between pregnant and non pregnant ewes. On the other hand, during the mid gestation (10 and 15 w.) a significant difference has been revealed between MP and PP ewes respectively with $p < 0.01$ and $p < 0.05$ respectively. However, during the 15th week, a very highly significant difference among ewes in the non pregnant group ($p < 0.001$) resulting probably from the ovarian activity especially in Mn-P than in Pn-P 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 Mn-P and Pn-P respectively.

DISCUSSION

Concerning the ration given to ewes, we noted that the supplement to the ration of basis (250g of concentrate/ewe/day) during this phase of preparation is weak comparatively to the required for the flushing. According to Nishina *et al.* [11], the restriction of diet during the periconceptual period by 30% induces cardiovascular problems due to vascular endothelial dysfunction, leading to elevation in blood pressure in the second half of gestation in twin fetuses without change in fetal weight. Low protein ration of cows exposes them to reproductive failure such as production of abnormal ova, change in uterine environment, feeble response of ovaries to gonadotropin and/or reduction of gonadotropin secretion, induces feeble expression or cessation of estrous and rises of repeat breeding [42]. However, it was asserted that protein supplement act directly as a neurotransmitter and on ovarian function or indirectly subsequent metabolism to enhance GnRH and LH secretion [43].

The values of total serum proteins in all groups are in the physiological limits as reported by Kaneko *et al.* [12] and Radostits *et al*. [13]. Generally, our results are in concordance with those obtained by Antunoviæ *et al* [14]; revealing higher concentrations of total serum proteins in non-pregnant ewes compared to the pregnant ones and those in lactation. In contrast to the observation of Caballero *et al.* [15], who noted the lowest value (65.0 g/L) at the start of the grazing period on stubble cereal than at the end (69.9 g/L). The highest values showed at the 10th w. and late pregnancy may be due to the haemoconcentration coinciding with the high temperature during summer [16]. Balikci *et al.* [17] reported the decrease in total serum protein on day 150 of gestation compared to other stages of gestation. This decline is associated to the fact that the fetus synthesizes its proteins from the amino acids provided by the mother and growth of fetus enhances exponentially reaching maximum levels during late pregnancy, particularly in muscles [18]. According to Braun *et al*. [19], the concentration of total proteins beyond the limit of 65g/L if it is associated with low corporal index, is highly indicative of a chronic inflammatory reaction. In other side, the values of serum total protein in pregnant O.D ewes are higher than those obtained in Makauit ewes at late pregnancy (145 day of gestation) by Batavani *et al.* [20], with 42.2±0.19 g/L. This significant decrease in serum protein levels could be a result of a rise in the mother's basal metabolic rate and increase in nutrient requirement of placenta and fetus.

The concentrations of serum albumin in all groups are not different from those described in published data [12, 13, 21]; however, they are higher than those obtained by Deghnouche *et al.* [22] in an arid zone of South- East Algeria in O.D ewes with 24.55±8.47 g/L and are almost similar to those obtained by Hamadeh *et al.* [23] in lactating and dry Awassi ewes subjected to daily watering with respectively 29.9 and 30.8 g/L. An increase of albumin is noted at the 150th day of pregnancy and at the 45th day post -partum by Balikci *et al.* [17]. According to Lynch and Jackson [5], there was no significant effect of feed restriction on serum albumin levels in pregnant ewes compared to those having a free-choice intake, in spite of a light rise of the values in the last ones. Shetaewi and Daghash (1994) cited by Balikci *et al.* [17] and Antunoviæ *et al*. [24] noted a decrease in the level of albumin and total protein during lactation in comparison to pregnancy. According to Piccione *et al.* [25] and Kaneko *et al*. [12], a progressive decrease of albumin and total protein during the second half of pregnancy is due to the increase of the transfer of nutrient requirements toward the fetal pool and mammary gland. The level of albumin reflects the storage capacity of the total protein when the diet contains low protein and in this case the urea is recycled to rumen via saliva and little nitrogen is lost [44].

The serum urea values are in accordance with those obtained by Balikci *et al*. [17], who reported lower values in late pregnancy (150 days) and at 45 days postpartum. According to Firat and Ozpinar [26] there is no significant difference between multiparous and primiparous ewes. However, the urea concentration in blood can be influenced by some factors such as the season. For instance; Antunoviæ *et al*. [27] observed an increase of levels in summer than in winter for ewes during late pregnancy and lactation. Uremia is higher in pregnant and lactating than in dry ewes [24, 22]. On the contrary, according to Khatun *et al.* [28] gestation induces an increase in uremia alongside the pregnancy course. In general, our values are in the normal intervals of those observed with effect of body condition score and the greater urea ($2 \geq$ BCS ≤ 3) [29]. The increase of urea concentration during lactation is due to catabolizing muscle protein when large bodily reserves are mobilized [24]. In ruminants, in case of nitrogenous deficit, the urea is recycled in the rumen via saliva and to a less extent via the ruminal partition where it is converted again into ammonia and can serve for the growth of the rumen bacteria [30, 31]. In our study, the increase of serum urea during the EP and 15w. periods is due probably to the consumption of young grass in spring and after rains (summer-autumn) especially in non-pregnant ewes.

The values of progesterone in pregnant ewes are higher than those obtained in O.D ewes (with2.77±0.31 and 2.31± 0.31(at 17 and 35 d) by Benyounes *et al*. [33] and (with 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)) by Benyounes *et al*. [32], in Corriedale ewes (with 1.41±0.21(0-6d); 4.0±0.87 (16-30d); 4.6±1.08 (60-75d); 10.81±2.85(91-105d); 13.33±2.43 (136d - lambing) and 0.26±0.03ng/mL (8-15d post-lambing)) by Ganaie *et al.* [8] and in flushing and non flushing Egyptian Barki ewes (with 3.1±0.6 vs 2.4±0.8 (dioestrus), 4.3±0.8 vs4.0±0.9(EP), 8.7±1.6 vs 7.6±1.9 (mid gestation) and 7.2±1.8 vs 6.4±2.00 (LP) [43]. The values noted are equivalent to those obtained by Bashandy, Mostafa and Rhaman [34] and by Özpinar and Firat [35] 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 progesterone (means during gestation in MP: 11.67±7.68 and PP: 9.07±6.78 ng/mL) are lower than those obtained by Manalu and Sumaryadi [36] 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. Progesterone is the main hormone involved in the maintenance of pregnancy and may play an important role in mediating the effect of nutrition on embryo development [37]; and that the high level of serum progesterone over the luteal phase of the estrous cycle after mating is indicative to high conception rates [38]. The reduction in pregnancy rates of ewes with peripheral progesterone concentrations above 5ng/mL on day 12 was probably due to direct effects of undernutrition rather than elevated progesterone. In other side, the increases in serum concentrations of progesterone are noted in response to feed restriction in sheep [8, 39, 40], cattle [41] and in non pregnant goat with high proportion of persisted corpus luteum [45].

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 variations in blood proteinic metabolites followed the nutritional level during the different periods; the values obtained ranged within the normal intervals and globally there was no significant difference between groups and period. The level of serum progesterone is maintained continuously elevated during pregnancy and decreased thereafter in early lactation. Also, there is an 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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