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Viability of Embryos and Fetuses in Relation with Backfat Depth of Ewes at Mating

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Abstract

NUTRITION plays a pivotal role in the reproductive performance of ewes. Ewes in optimal body condition tend to have larger litters, higher lamb survival rates, and heavier lambs at weaning. While obese animals may suffer a reproductive insufficiency. Therefore, monitoring the body condition of ewes throughout their reproductive cycle is essential for maximizing flock productivity. The present work aimed to investigate the effect of rump fat depth of subtropical ewes (n = 62) at mating on their embryonic and fetal development. Pregnancy rates, reproductive wastages, level of leptin hormone, and embryonic and fetal measurements, were studied. Data were analyzed using one-way analysis of variance to determine statistically significant differences among backfat depth groups (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; >2mm). The lowest rump fat depth group had the lowest values for pregnancy rate, ewes lambed and lambs born per ewes conceived and leptin concentrations; the highest reproductive wastage was attained. Higher fetal measurements (crown-rump length, the largest placentome diameter, biparietal diameter, chest depth and trunk diameter) were observed for ewes having a backfat depth of more than 1.5 mm. However, no significant differences were observed for ewes with backfat thicknesses of >1.5 to ≤2 mm and those having >2 mm regarding all the measured traits. Therefore, controlling the rump fat depth of ewes before mating is so important for enhancing the investment of sheep flocks by reducing feeding cost and to get higher proportion of born lambs with higher weights.

Keywords: Back fat, pregnancy rate, ewes, embryonic and fetal measurements, leptin.

Introduction

Enhancing the profitability of sheep enterprises requires increasing the lamb crop. It can be achieved by raising ewes with an optimal body condition score. Using ultrasonography, measuring the backfat thickness of ewes is a good quantified indicator to express body condition score [1]. Backfat thickness is used to measure the amount of fat found on the back of an animal, between the skin and muscle tissues, and it is considered an important indicator reflecting the content of adipose tissue present [2]. Since predicting backfat thickness by ultrasonography is efficient in small ruminants [3-7].

Accurately predicting gestational age in sheep, especially in cases of uncertain mating dates, is crucial for various reasons among the breeding sectors including producers, veterinarians, and researchers [8]. It allows the breeders to start optimal management measures including feeding, housing construction, lambing, and time of lactation cessation in dairy farms, in addition to clinical interventions and inducing parturition. Additionally, researchers rely on accurate gestational age determination when using sheep as models in biomedical studies [9].

Ultrasound measurements of fetal bones provide a non-invasive accurate method for estimating gestational age in sheep, making it an essential tool for improving flock health, optimizing production, and advancing scientific research. [7], in addition to its involvement in effectively, measuring embryo and fetal mortality at 20-50 days of pregnancy [1,10,11]. Particularly, the reproductive performance of sheep is improved during the first 50 days after mating by using the rearing technologies as reported by [10]. Also, sheep carcasses are positively and highly correlated with in-vivo ultrasound measurements of backfat thickness over the lumbar region or the end part of the thoracic vertebrae [3,5,6].

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Between ovulation and weaning, it was recorded 30-43.3% of reproductive wastage in ewes [12-14]. Where materno-fetal stress during pregnancy period increases fetal death, aberrant metabolism, inefficient growth and low birth weight [15]. Therefore, managing body reserves through their mobilization and accretion has an important role in livestock sustainability [16], making them more adapted to variable environments, leading to acquiring high economic importance [17].

However, strong relationships were reported between the backfat thickness of the subtropical ewes and their reproductive and productive performance [1]. Thus, the effect of backfat depth of subtropical Egyptian ewes on their embryos and the fetus viability was investigated in this study.

Material and Methods

Before conducting this study, the protocol followed the Animal Care and Use Committee of the Medical Research Ethical Committee (MREC) of the National Research Centre. The present study was carried out at the experimental farm of the animal production department, Faculty of Agriculture, Cairo University, Giza, Egypt, in the summer season, to investigate the effect of backfat depth on embryonic and fetal development in Barki and Rahmani subtropical sheep.

Animals and management

Two subtropical Egyptian sheep breeds, Barki (n = 33) and Rahmani (n = 29) were used. Age, initial body weight, body condition score and backfat depth, of ewes, are presented (Table 1). Ewes were allotted to 4 groups, according to their backfat depth (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; > 2mm) at the terminal end of the thoracic vertebrae. Ewes received the same regimen according to their feeding allowances that varied according to the stage of pregnancy [18]. Females were maintained in semi-shaded pens, where drinking water was freely available. Clover, clover hay, a concentrate mixture (13% CP and 0.65 kg TDN) and wheat straw were used in the diet formulation. Animals were synchronized for estrus using two intramuscular injections of 250 µg Cloprostenol (PGF2α, Estrumate, Coopers Co., England) at an 11-day interval. Simultaneously with the second injection, fertile rams of the same breed were introduced to ewes in a ratio of 1:8.

Blood sampling and hormonal assay

Blood samples were collected via jugular vein puncture at the time of breeding (Day 0) and on Day 45 of pregnancy. Blood samples were allowed to clot and sera were separated by centrifugation at 3000 rpm for 15 min. Sera were stored at -20°C until hormonal assay. Mutli-Species Leptin RIA Kit (Linco Research) was estimated. The limit of sensitivity, intra- and inter-assay coefficients of variation was 1.0 ng/ml, 2.8% and 8%.

Measured traits

Reproductive traits at Days 17, 45, 75 (mid-pregnancy) and 140 of pregnancy (late pregnancy) their pregnancy rate (PR); numbers of embryonic vesicles, embryos or fetuses and reproductive wastage (RW) were measured. Numbers of ewes lambed per ewes conceived (EL/EC) and lambs born per ewes conceived (LB/EC) were also calculated. PR was considered as the number of ewes pregnant per ewes mated at Days 17, 45, mid-pregnancy and late pregnancy. RW was measured as the difference between ovulation rate (OR) and the number of embryonic vesicles, embryos or fetuses, concerning OR. The OR was measured by counting the number of corpora lutea at days 7-10 after mating. Backfat depth, OR, pregnancy diagnosis, embryonic measurements and fetal measurements were evaluated by ultrasonography, using a 6–8 MHz real-time, B-mode linear array ultrasound scanner (Model: Scanner 100 LC, Pie Medical Company, Maastricht, Netherlands). The crown-rump length (CRL, all embryo length) was measured at Days 25 and 45 of pregnancy. Then, at Day 75 of pregnancy, the biparietal diameter (BPD, the distance between the two parietal bones of the head), chest diameter (CHD, the distance between thoracic vertebrae and the sternum) and trunk diameter (TRD, the distance from the vertebral column to the umbilicus) were also measured, in addition to the mean diameter of the largest three placentomes.

Statistical analysis

Data were subjected to a one-way analysis of variance to determine statistically significant differences among backfat depth groups (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; >2mm). The breed of ewes was considered a covariate in the statistical model. Data of the measured traits were analyzed using the GLM procedure. The Least Squares Means (LSM) were obtained for the traits of CRL, fetal measurements and leptin level. While the PR, RW, EL/EC and LB/EC traits were analyzed using the chi-square CATMOD procedure. The Duncan's Multiple Range Test was used to detect differences among means. The significance level was set at $P < 0.05$ [19].

Results

The effect of backfat depth on pregnancy rate of ewes throughout the different pregnancy periods is presented in Figure 1. No significant differences were observed among groups for pregnancy rate at Day 17 post-mating, while the significant differences were present at the rest of pregnancy periods. Where the lowest values for pregnancy rates were recorded for the lowest backfat depth ewes ($P < 0.05$), and it increased with increasing their backfat depth; the significance level was absent among the other three groups. The effects of ewes' backfat depth on numbers of embryonic vesicles, embryos or fetuses throughout pregnancy stages are found in Figure 2. The lowest numbers ($P < 0.05$) of embryonic vesicles at Day 17, fetuses at Days 45, 75 and 140 were observed in pregnant ewes had the lowest backfat depth. The highest death rates for embryos and fetuses were recorded for ewes had the lowest backfat depth, particularly at the last two-thirds of pregnancy term (Figure 3). While the significance level was not attained between the highest two backfat depth groups.

Concerning the serum leptin concentration in the experimental groups, Table (2) showed that, the lowest leptin concentration was recorded in ewes with the minimal backfat thickness. While that with highest backfat had the highest leptin concentrations. Lean pregnant ewes, at Day 45 of pregnancy, of backfat thickness ≤ 1mm had similar leptin to their class at mating and still had the lowest leptin concentrations compared to other pregnant classes. However, only the group of the maximum backfat thickness (>2mm) had higher ($P < 0.05$) leptin level than the ≤ 1mm group.

Regarding to effect of backfat depth of ewes on their embryonic and fetal measurements, the data of table (3) pointed out that, the crown–rump length of embryos did not differ significantly for the different backfat groups, neither on Day 25 nor Day 45 of pregnancy. The diameter of the largest placentomes was significantly higher for the higher backfat thickness groups. Ewes with backfat from >1mm to ≤1.5 mm and from >1.5 to ≤ 2 mm had similar placentome size.

Bi-parietal diameter of fetuses at mid-gestation was significantly lower in ewes had the lowest backfat thickness (Table 3); however, the other three backfat depth groups did not differ significantly from each other. Both chest diameter and trunk diameter of fetuses at mid-gestation were significantly higher with increasing backfat depth of ewes.

The effects of ewes' backfat depth on ewes lambed per ewes conceived (EL/EC) and lambs born per ewes conceived (LB/EC) are presented in Figure 4. The highest values were those of ewes having backfat depth of more than 1.5 mm and the lowest were for those having 1 mm or less ($P < 0.05$).

Discussion

The current research examined the effect of backfat depth on embryonic and fetal development in subtropical Egyptian sheep. The backfat depth of ewes and its relation with reproductive and productive performances was already studied on Egyptian sheep [1] without focusing on measurements of embryos and fetuses, is the main topic of this study.

The results [Figure 1] indicated no significant differences in the pregnancy rate at Day 17 post-mating among the backfat depth groups, significant differences were attained at the rest of the pregnancy stages. Hashem and El-Zarkouny [20] reported an average conception rate of 60% in the Egyptian Barki and Rahmani ewes at Day 35 post-mating, which is lower than that observed in the present study.

Most of ewes conceiving [93-100%], with a backfat depth of more than 1.5 mm, ultimately giving birth, compared to only 58% of those having the lowest backfat depth [≤1 mm] [Figures 1 and 4]. Higher proportions [$P < 0.05$] for lambed ewes and born lambs were previously observed for higher backfat thickness Egyptian ewes compared to the lower backfat ones [1]. Similarly, Abdel-Mageed [21, 22] mentioned higher [$P < 0.05$] conception rates in Egyptian ewes with a moderate and a highly body condition score, that's consistent with results of the present study. Actually, raising ewes in moderate adiposity is very important for enhancing its pregnancy rate [23]. However, ewes had moderate adiposity in the present study had the highest [$P < 0.05$] number of embryonic vesicles at Day 17, in addition to fetuses at Day 45, mid- and late pregnancy [Fig. 2]. Actually, adequate body reserves are needed during pregnancy and lactation [24, 2]. Where a reduction in body fat is associated with higher prenatal losses [25], in addition to low reproductive efficiency in farm animals [26]. Also, it was observed an adverse effect on milk yield and reproductive performance for ewes having a reduction in body condition score [27; 15]. At the same context, Hu and Yan [28] found that, maternal backfat thickness had different effects on characteristics of the newborn piglets, in addition to its effect on maternal placental inflammation, lipid metabolism, and oxidative stress. Therefore, body reserve changes must be quantified during the reproductive cycle of ewes [29].

The reproductive wastage at Day 17 of pregnancy, in the present study [Figure 3], ranged between 10 and 33 % of ova shed. While it reached 61% at late pregnancy in ewes having ≤ 1mm backfat depth compared to only 26% for those having > 1.5 mm backfat depth. Hashem and EL-Zarkouny [20] observed embryonic loss ranging between 25 and 55 % in Barki and Rahmani ewes at Day 35 post-mating. However, 30-43.3% of reproductive wastage was recorded in ewes between ovulation and weaning [12; 13; 14].

Placentomes, the functional units of the ruminant placenta, are formed by the interdigitation of maternal endometrial caruncles and fetal cotyledons [30; 31]. Ruminants like cattle, sheep, and goats typically have 75 to 125 placentomes scattered throughout the uterus. Each placentome consists of a maternal caruncular component [CAR] and a fetal cotyledonary component [COT]. Both CAR and COT tissues are highly vascularized, with blood vessels interdigitating at the fetal-maternal interface to facilitate efficient nutrient and gas exchange [32]. As the fetus grows during gestation, the placentome undergoes increased vascularization to meet the rising demands for nutrients and oxygen [33]. Significant increases were found in total placentomes weight, fetal weight, crown-rump length [34], thoracic girth and abdominal girth [35] at mid-pregnancy for obese ewes than the controls, which agree with results of the present study related to fetal measurements [Table 3]. In the present study, no significant differences were observed in CRL among the different backfat depth groups, while BPD had lower values in the lowest backfat depth one. However, no significant effect was observed for pre-mating nutritional treatments, litter size or fetal sex on the biparietal diameter of the fetus during the first half of pregnancy [7].

Leptin is an adipocyte-secreted hormone that regulates food intake, energy expenditure, and body weight [36]. Kaplan [37] stated that the leptin gene has many important biological functions that affect the economically important traits of livestock animals. Leptin has been implicated in the regulation of appetite, energy expenditure, whole-body energy balance and reproduction [38, 39, 40, 41]. In sheep, there are high correlations between body fat mass and leptin levels [42, 43, 17]. Since leptin level was higher in the highly BCS pregnant ewes compared to the lower ones at Day 50 of pregnancy [44] that agrees with the results of the present study. Increasing leptin levels still higher in the high BCS ewes throughout the last three weeks of pregnancy as reported by Alvarez-Rodríguez [45]. However, the increase of leptin concentrations in early pregnant fat-tailed ewes of this study with the higher backfat thickness confirms the positive association of backfat thickness and leptin concentrations in adult ewes, whatever pregnant or non-pregnant.

The higher mid-gestation placentome diameter, fetal chest diameter and trunk diameter of the higher backfat thickness and leptin concentrations observed in ewes of this study could be due to the positive relationship existed between leptin expression in fetal adipose tissue and fetal weight in sheep [46]. Adipose tissue not only produces many substances including leptin, but these biomarkers could be related to intrauterine growth retardation and large for gestational age in pregnant females [47, 48]. At mid-gestation, intrauterine growth restriction lambs remained smaller than normal birth weight lambs at necropsy and this growth restriction was referred to due to placental insufficiency. Vautier and Cadaret [48] stated that maternal stress maintained during early to mid-gestation, when peak placental growth is taking place, is more likely to cause placental stunting than during late gestation when it may instead lead to a direct fetal insult.

Conclusion

As a conclusion for this paper, controlling the backfat depth of ewes before mating to be moderate (1.5-2 mm) is so important to improve the reproductive performance of ewes via increasing the pregnancy rate and reducing reproductive wastage to get a higher proportion of lambs born having higher weights.

Acknowledgments

Not applicable.

Funding statement

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This protocol followed the regulations of the Animal Care and Use Committee of the Medical Research Ethical Committee (MREC) of the National Research Centre.

TABLE 1. Mean±SD (Standard deviation) of age, body weight (BW), body condition score (BCS) and backfat depth (BF) of subtropical Egyptian ewes.

Backfat depth	N	Age (years)	BW (kg)	BCS (unit)	BF (mm)
≤ 1mm	16	3.36±0.93	32.68±3.00	2.39±0.31[a]	0.91±0.11[a]
>1 - ≤ 1.5 mm	15	3.19±1.26	34.92±5.45	2.66±0.23[ab]	1.28±0.08[b]
>1.5 - ≤ 2 mm	16	3.21±1.17	36.54±3.58	2.78±0.24[ab]	1.74±0.15[c]
> 2mm	15	3.38±0.76	39.85±4.69	3.02±0.37[b]	2.48±0.30[s]
P-Value	62	0.995	0.290	0.144	0.0001

Means with different superscripts in the same column (a,b,c,d)are significantly different at P<0.05

TABLE 2. Mean ± SEM (standard error of the mean) of leptin level (ng/ml) as affected by backfat depth in subtropical Egyptian ewes.

Backfat depth/mm	Mating	Day 45
≤ 1.0	2.85± 0.41[a]	2.97± 0.85[a]
>1.0 to ≤ 1.5.0	3.10± 0.40[a]	3.50 ± 0.85[ab]
>1.5 to ≤ 2.0	3.27 ± 0.38[a]	4.06± 0.74[ab]
> 2.0	4.09± 0.43[b]	4.65± 0.76[b]
P-value	0.027	0.125

Means with different superscripts in the same column (a,b)are significantly different at P<0.05

TABLE 3. Mean ± SEM of embryonic crown-rump length (CRL) and fetal measurements as affected by backfat depth in subtropical Egyptian ewes.

Backfat depth/mm	CRL (cm)		Fetal measurements at mid-pregnancy (cm)			
	Day 25	Day 45	PLD	BPD	CHD	TRD
≤ 1.0	1.30±0.05	3.87 ±0.09	2.30±0.22[a]	3.27±0.20[a]	3.30±0.38[a]	3.84±0.39[a]
>1.0 to ≤1.5	1.33±0.05	3.79 ±0.08	2.76±0.20[b]	4.04±0.18[b]	3.89±0.34[ab]	4.51±0.35[ab]
>1.5-≤2.0	1.28±0.05	3.85 ± 0.08	2.78±0.22[b]	3.94±0.20[b]	4.14±0.38[b]	5.09±0.38[bc]
> 2.0	1.33±0.06	3.86 ± 0.09	3.09± 0.22 [b]	4.07±0.21[b]	4.41±0.47[b]	5.73±0.40[c]
P-value	0.606	0.671	0.013	0.003	0.044	0.02

Means with different superscripts in the same column (a,b,c) are significantly different at P<0.05, Fetal measurements include PLD (placentome diameter), BPD (bi-parietal diameter), CHD (chest diameter) and TRD (trunk diameter).

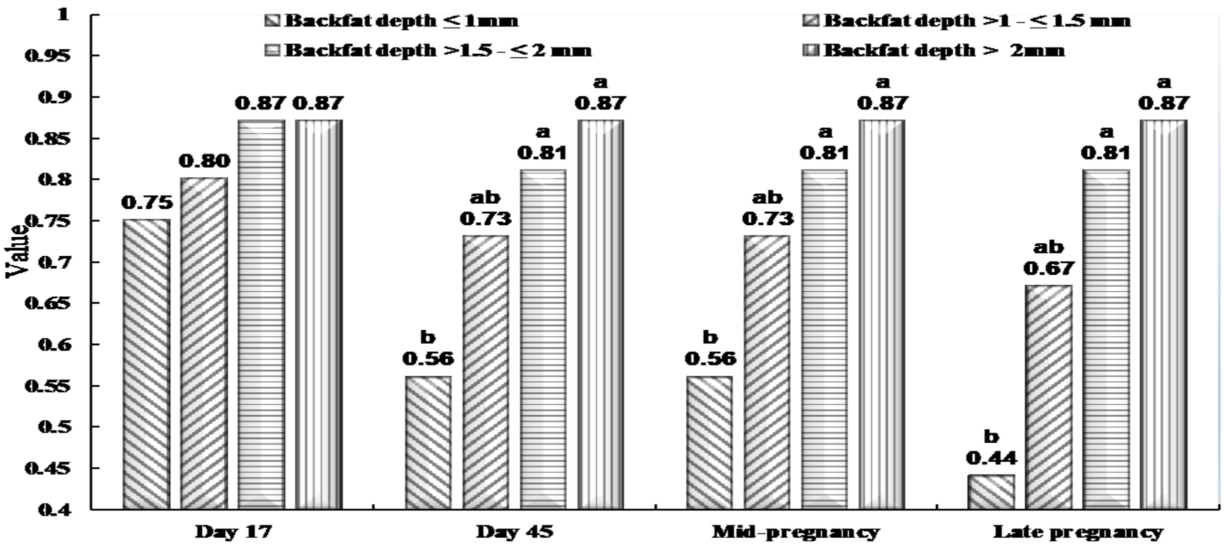

Fig. 1. Means of pregnancy rates at different times of pregnancy as affected by backfat depth of ewes at mating (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; > 2mm). Columns with different letters differ significantly at P < 0.05. Pregnancy rate was considered as the number of ewes pregnant per ewes mated.

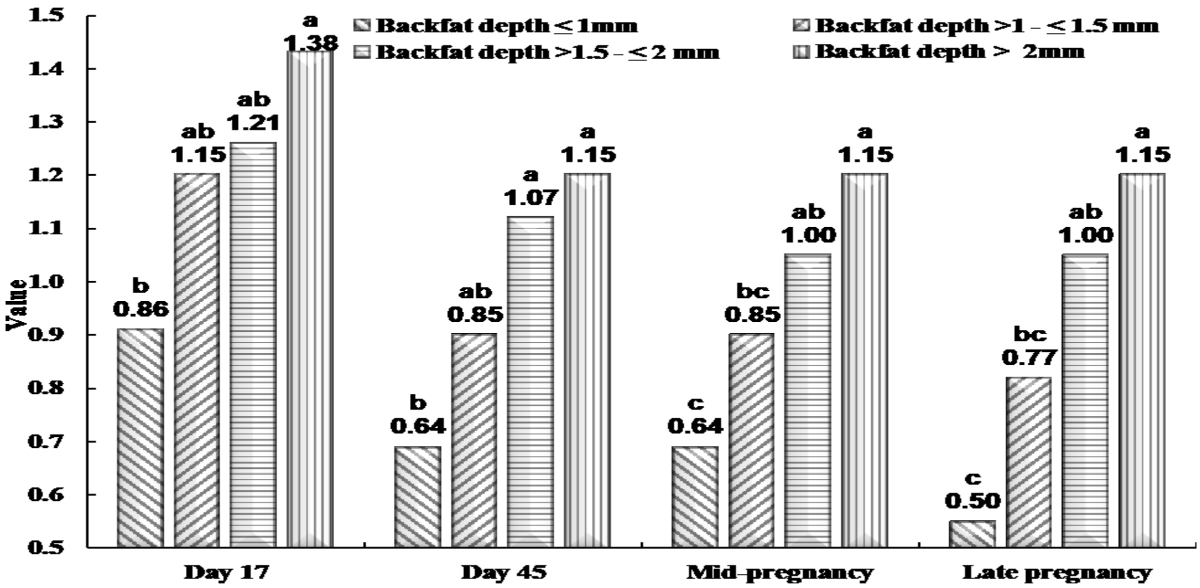

Fig. 2. Means per ewes conceived for numbers of embryonic vesicles at Day 17 and fetuses at Day 45, mid- and late pregnancy as affected by backfat depth of ewes at mating (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; > 2mm). Columns with different letters differ significantly at P < 0.05.

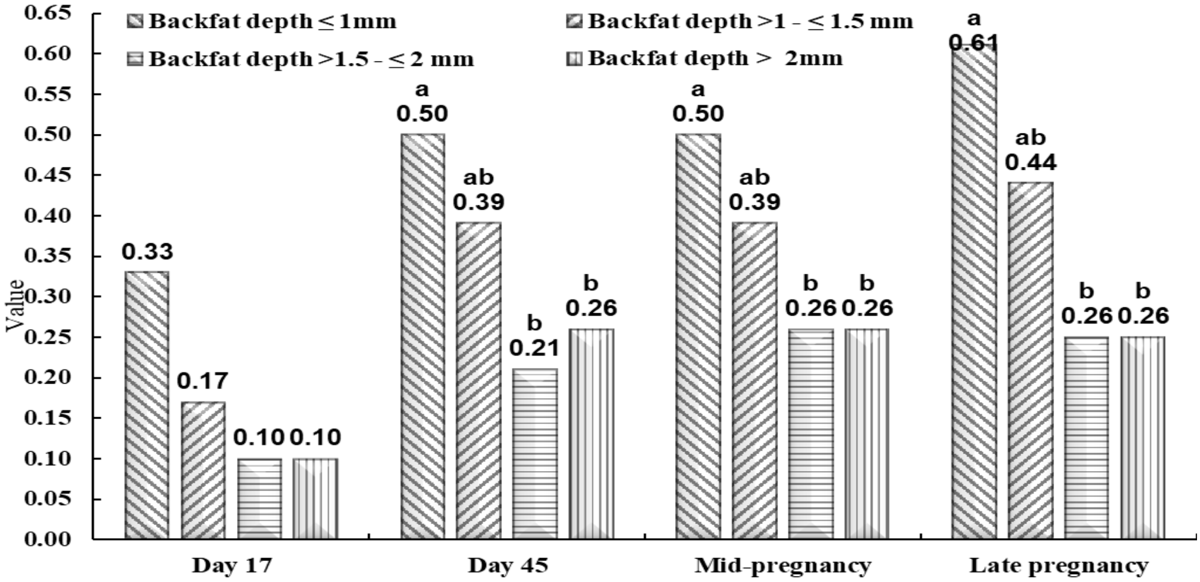

Fig. 3. Means of reproductive wastages at different times of pregnancy as affected by backfat depth of ewes at mating (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; > 2mm).Columns with different letters differ significantly at P < 0.05. Reproductive wastage was measured as the difference between ovulation rate and number embryonic vesicles at Day 17 or number of fetuses at Day 45, mid- and late pregnancy, in relation to OR.

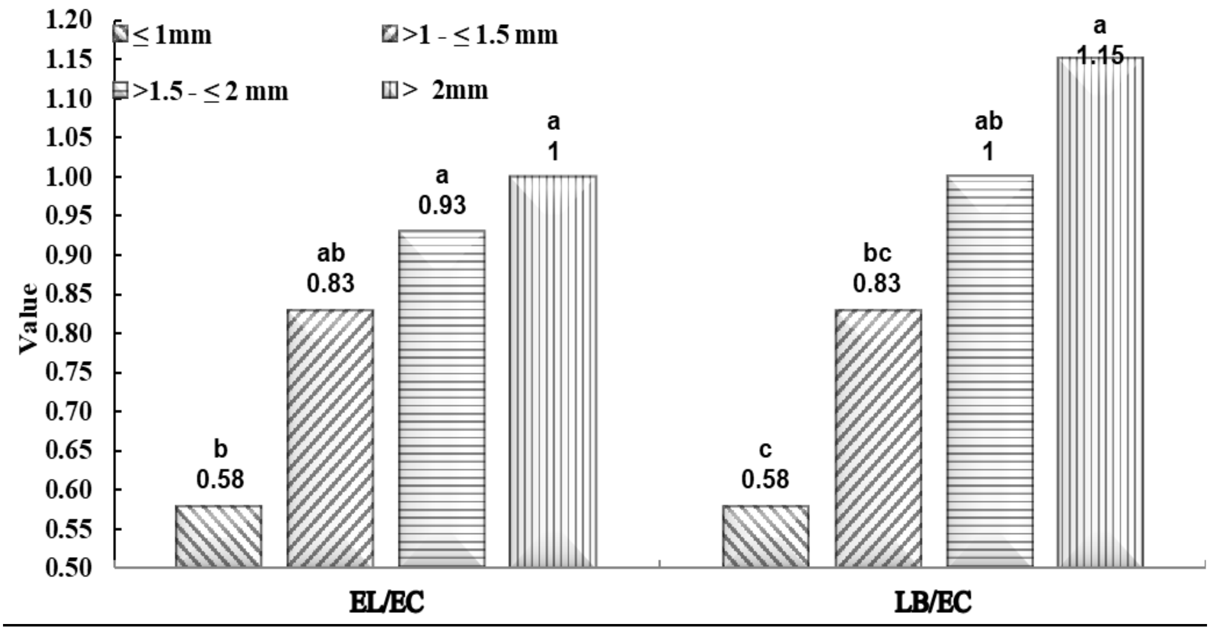

Fig. 4. Means of numbers of ewes lambed per ewes conceived (EL/EC) and lambs born per ewes conceived (LB/EC) as affected by backfat depth of ewes at mating (≤1 mm; >1 - ≤ 1.5 mm; >1.5 - ≤ 2 mm; > 2mm). Columns with different letters differ significantly at P < 0.05.

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حيوية النطف و الاجنة و علاقاتها بسمك طبقة الدهون في النعاج عند التزاوج

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الملخص

تلعب التغذية دور محوري في تحسين الكفاءة التناسلية للنعاج. وترتبط حالة الجسم القصوى مع زيادة عدد الحملان المولودة واستمرارها في النمو وحتى الوصول لاعلى اوزان عند الفطام. بينما تميل الحيوانات السمينة من اختلال كفاءتها التناسلية لذا فان تقييم حالة الجسم للنعاج خلال دورة التناسل اساسي لتعظيم انتاجية القطيع. و يهدف هذا البحث لمناظرة تاثير سمك طبقة الدهون بظهرالنعاج المحلية تحت اجواء شبه حارة و عددها 62 نعجة عند التزاوج على نمو النطف و الاجنة و تم دراسة معدلات الحمل و الفاقد من دورة التناسل و مستويات هرمون الليبتين و قياسات النطف و الاجنة و تم تحليل البيانات باستخدام معامل التباين البسيط ذو الاتجاه الواحد بعد تحديد تاثير سمك طبقة الدهون بالظهر لتقسيم النعاج للاربعة مجموعات(اقل من 1مم و اكبر من 1مم لاقل من 1.5مم و اكبر من 1.5مم لاقل من 2مم و اكبر من 2 مم) و اتضح من النتائج ان انخفاض سمك طبقة الدهون اقترن بانخفاض معدلات الحمل و عدد امهات النعاج و عدد الحملان المولودة للنعاج الملقحة و تركيزات هرمون الليبتين مع ارتفاع الهدر او الفقد التناسلي. و قد اقترنت زيادة طبقة الدهون عن 1.5 مم بزيادة بقياسات الاجنة مثل طول الجنين من الراس للذيل و حجم اقراص المشيمة و المسافة بالجمجمة بين عظمتي خلف الاذن و قطر الصدر و الوسط من عند السرة و . كما لم تظهر اي فروق معنوية بين النعاد ذات سمك طبقة الدهون اكبر من1.5 و اقل من 2 مم و تلك التي كانت سمك طبقة الدهون بها اكبر من 2 مم بالنسبة للقياسات السابقة. لذا ف التحكم بسمك طبقة الدهون في النعاج قبل التزاج مهم جدا لتحسين الاستثمار بالاغنام لما سيترتب عليه تقليل تكلفة التغذية و الحصول على اعى نسب من المواليد ذات اعلى اوزان

الكلمات الدالة: دهون الظهر ، معدل الحمل ، االنعاج ، قياسات الاجنة ، الليبتين.