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Asian Journal of Animal and Veterinary Advances



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Does Parity and Nutrition in Early Pregnancy Affect Viability of Embryos in Both Rahmani and Barki Egyptian Sheep?

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ABSTRACT

The aim of this study was to investigate the effects of sheep breed, parity and post-mating nutrition on embryo wastage in early pregnancy. The experimental design was completely randomized and the groups of ewes arranged factorially (2×2×3) according to the breed (Rahmani or Barki), parity of the ewes (nulliparous or parous) and post-mating nutritional regimen through the first 45 days of pregnancy (70% of the maintenance requirements, or 130% of the maintenance requirements). Rahmani ewes recorded significantly higher estimates than Barki ewes in crown-rump length at days 25 and 45 of pregnancy. At day 45 of pregnancy, both parity and post-mating nutrition significantly affected body weight, body condition score and backfat thickness of ewes. A clear reduction in pregnancy rate estimates, at day 45 of pregnancy, were recorded in the under- and the over-nourished ewes compared to the controls. A double-fold of embryonic wastage was recorded in the over-nourished ewes and three folds in the under-nourished ones at day 45 of pregnancy in comparison with the medium-nourished ewes. Crown-rump length of embryos was higher in the over-nourished animals. The under-nourished ewes had higher P₄ level at days 25 and 45 of pregnancy, while the over-nourished ones recorded lower estimates than the control ewes. Therefore, it is important to control the feeding allowance of ewes in early pregnancy to be around the maintenance requirements to get a considerable proportion of viable embryos.

Key words: Sheep, breeds, parity, postmating nutrition, embryo wastage

INTRODUCTION

Production of viable new-born lambs is the main target of sheep breeders. It can be achieved by reducing death rate of embryos and fetus in pregnant ewes. Since embryonic and fetal mortality contribute to a large economic loss in sheep (Dixon *et al.*, 2007). Therefore, embryo survival is a major factor affecting production and economic efficiency in all systems of ruminant milk and meat production (Diskin and Morris, 2008). Ataman *et al.* (2013) concluded that, ewe's embryonic losses are observed within the first 3 weeks of pregnancy. Losses before the gestational 18th day, when the embryo is not attached to the uterine epithelium are referred to as "early embryonic death" (Hafez and Hafez, 2000), while Dixon *et al.* (2007) stated it to be occurred before the 25th day of pregnancy and the period between days 25 and 45 is considered as "late embryonic death". Late embryonic and foetal death could be detected effectively with a single ultrasonography between the 20th and the 50th gestational days (Yotov, 2012). Many factors are responsible for this embryonic

and fetal mortality like body weight, body condition score, nutrition, breed, parity, ovulation rate, number of embryos, stage of pregnancy and progesterone level. This study investigated the effects of breed, parity and post-mating nutrition on embryo wastage in early pregnancy of two Egyptian sheep breeds, Rahmani and Barki.

MATERIALS AND METHODS

The present study was carried out at the experimental farm of the Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt, in summer season, to investigate the effects of breed, parity and post-mating nutrition on embryo wastage in early pregnancy. The latitude of this location is 30°N and the longitude is 31°E.

Animals and management: Two Egyptian sheep breeds (Rahmani [R] and Barki [B], n = 98, Table 1) were used in the trial. The values of initial body weight, age, body condition score, backfat thickness and back muscle depth of ewes of the two breeds were presented in Table 2. Immediately after mating, ewes within breed (Rahmani and Barki) and parity (nulliparous or parous), were allocated into three groups according to nutritional regimen that they consume daily: (1) 70% of Maintenance Requirements (MR), (2) MR and (3) 130% of MR. The nutritional regimen continued through the first 45 days after mating. While through the rest of the pregnancy period, the three groups had the same regimen according to their feeding allowances that varied according to the stage of pregnancy (NRC., 1985). Females were maintained in semi-shaded pens, where drinking water was freely available. Clover hay, a concentrate mixture (13% CP and 0.65 kg TDN) and wheat straw were used in the diet formulation. The concentrate mixture consisted of 55% yellow corn, 20% wheat bran, 10% cottonseed cake, 10% soybean meal, 2% lime stone, 1.5% common salt, 1% minerals mixture, 0.4% sodium bicarbonate and 0.1% AD3E vitamins. Animals were synchronized for oestrus using two intramuscular injections of Estrumate (PGF2 α , each of 1 mL or 250 μ g Cloprostenol, Coopers Co., England) at an 11 day interval. Simultaneously with the 2nd treatment of Estrumate, fertile rams of the same breed were introduced in a ratio of 1:8.

Table 1: No. of ewes per treatment used in a study of the effects of breed, parity and nutritional level during the first 45 day post-mating

Parameters	Sheep breeds		Total
	Rahmani	Barki	
Parity			
Nulliparous	15	21	36
Parous	29	33	62
Post-mating nutrition			
0.7 of feed needs	15	18	33
Feed needs	13	15	28
1.3 of feed needs	16	21	37

Table 2: Mean \pm SD of measured traits (BW, BCS, BF, BM and age) of two breeds of Egyptian ewes

	N	BW (kg)		Age (years)		BCS (unit)		BF (mm)		BM (mm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rahmani	44	36.70	5.13	3.43	1.11	2.50	0.43	1.20	0.35	1.70	0.31
Barki	54	33.85	6.23	3.30	1.54	2.68	0.45	1.44	0.51	1.88	0.36

BW: Body weight, BCS: Body condition score, BF: Backfat thickness and BM: Back muscle depth

Measured traits: The traits measured were Body Weight Difference (BWD), Body Condition Score Difference (BCSD), backfat thickness difference (BFD), Back Muscle Depth difference (BMD), pregnancy rate at days 18 (PR18), 25 (PR25) and 45 (PR45) of pregnancy, embryo wastage at days 18 (EW18), 25 (EW25) and 45 (EW45) of pregnancy, crown-rump length at days 25 (CRL25) and 45 (CRL45) of pregnancy, progesterone level at days 7 (P₄7), 25 (P₄25) and 45 (P₄45) of pregnancy. The measurements of BWD, BCSD, BFD and BMD were calculated as the differences of estimates between the beginning (mating) and the end of the nutritional treatment (45 days after mating). Pregnancy rate was considered as the number of ewes pregnant per ewes mated. Embryo wastage was measured as the difference between Ovulation Rate (OR) and number of embryonic vesicles at day 18 or number of embryos at days 25 and 45 of pregnancy, in relation to OR. The OR was measured by counting the number of corpora lutea at day 7 after mating. The measurements of backfat thickness, OR, pregnancy rate, embryo wastage and crown-rump length were measured by ultrasonography, using a 6-8 MHz real-time, B-mode linear array ultrasound scanner (Model: Scanner 100 LC, Pie Medical Company, Maastricht, Netherlands).

Blood sampling: Blood samples (5 mL) were withdrawn from the jugular vein at days 7, 25 and 45 after mating. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C till progesterone concentration assessment. Determination of P₄ in plasma was carried out by ELISA technique.

Statistical analyses: The experimental design was completely randomized and the groups of ewes arranged factorially (2×2×3), according to the breed (Rahmani or Barki), parity of the ewes (nulliparous or parous) and post-mating nutritional regimen (70% of MR, N1, MR, N2 or 130% of MR, N3) being the main effects. The interactions were also considered. Data of the measured traits were analyzed using the GLM procedure and the Least Squares Means (LSM) were obtained for the traits of BWD, BCSD, BFD, BMD, CRL25, CRL45, P₄7, P₄25 and P₄45. While the traits of PR18, PR25, PR45, EW18, EW25 and EW45 were analyzed using the chi-square CATMOD procedure. The Tukey's studentized range (HSD) test was used to detect differences among means. The significance level was set at p<0.05 (SAS Institute Inc., 2004).

RESULTS

For most traits, no interactions were recorded among breeds, parity and post-mating nutritional treatment. Therefore, the results only highlighted the impact of the main effects on the studied traits.

Breed did not significantly affect the studied traits (Fig. 1, Table 3) except crown-rump length at day 25 (CRL25) and crown-rump length at day 45 (CRL45), where Rahmani ewes showed higher (p<0.01) estimates (1.44 and 4.09 mm, respectively) than Barki ewes (1.22 and 3.70 mm, respectively).

Parity of ewes affected Body Weight Difference (BWD), Body Condition Score Difference (BCSD) and Backfat Thickness Difference (BFD) as presented in Table 4, while it had no significant effect on the rest of the studied traits (Fig. 2, Table 4). Females of the 1st parity lost 0.93 kg, 0.04 unit and 0.02 mm in their BW, BCS and BF, respectively, while the parous ewes acquired 0.47 kg, 0.06 unit and 0.05 mm, respectively.

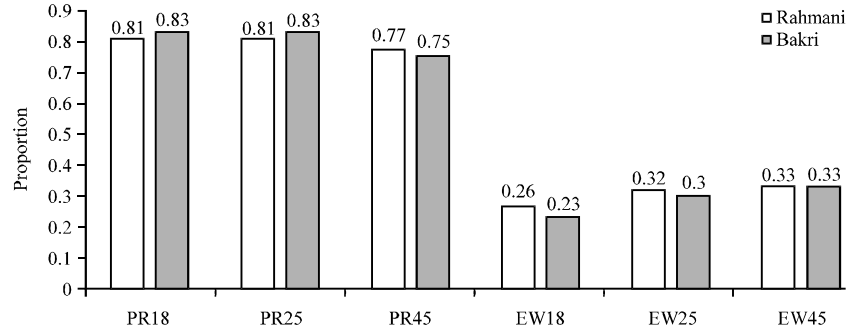


Fig. 1: Means of Pregnancy Rate (PR) and Embryo Wastage (EW) at days 18, 25 and 45 after mating in Rahmani and Barki ewes. Pregnancy rate was considered as the number of ewes pregnant per ewes mated. Embryo wastage was measured as the difference between Ovulation Rate (OR) and number of embryonic vesicles at day 18 or number of embryos at days 25 and 45 of pregnancy, in relation to OR

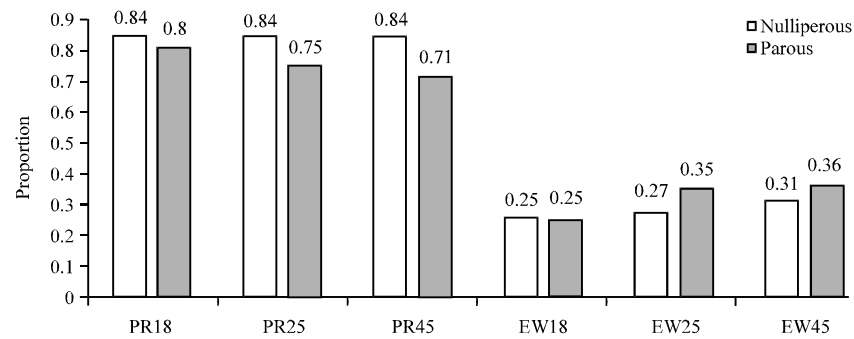


Fig. 2: Means of Pregnancy Rate (PR) and Embryo Wastage (EW) at days 18, 25 and 45 after mating in nulliparous and parous ewes. Pregnancy rate was considered as the number of ewes pregnant per ewes mated. Embryo wastage was measured as the difference between Ovulation Rate (OR) and number of embryonic vesicles at day 18 or number of embryos at days 25 and 45 of pregnancy, in relation to OR

Table 3: LSM±SE of measured traits (BWD, BCSD, BFD, BMD, CRL and P₄) of Rahmani and Barki ewes

Parameters	Rahmani	Barki
BWD (kg)	-0.27±0.18	-0.18±0.16
BCSD (unit)	0.03±0.03	0.00±0.02
BFD (mm)	0.05±0.03	-0.01±0.02
BMD (mm)	0.02±0.03	-0.02±0.02
CRL25 (mm)	1.44±0.02 ^a	1.22±0.02 ^b
CRL45 (mm)	4.09±0.04 ^a	3.70±0.04 ^b
P ₄ 7 (ng mL ⁻¹)	3.64±0.13	3.71±0.12
P ₄ 25 (ng mL ⁻¹)	3.77±0.13	3.72±0.12
P ₄ 45 (ng mL ⁻¹)	3.82±0.16	3.75±0.15

Columns with different LSM superscripts differ significantly from each other (p<0.05), D: Refers to the difference between the measurements at mating and day 45 after mating, CRL was measured at days 25 and 45 of pregnancy, P₄ was measured at days 7, 25 and 45 of pregnancy, BWD: Body weight difference, BCSD: Body condition score difference, BFD: Backfat thickness difference, BMD: Back muscle depth difference, CRL: Crown-rump length and P₄: Progesterone level, R: Rahmani and B: Barki ewes

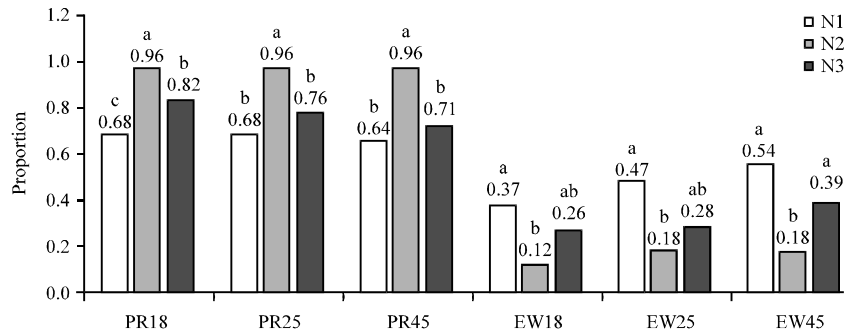


Fig. 3: Means of Pregnancy Rate (PR) and Pregnancy Wastage (EW) at days 18, 25 and 45 after mating in ewes fed 0.7 (N1), 1 (N2) and 1.3 (N3) of the recommended post-mating feed needs. Columns with different letters differ significantly from each other ($p < 0.05$). Pregnancy rate was considered as the number of ewes pregnant per ewes mated. Embryo wastage was measured as the difference between Ovulation Rate (OR) and number of embryonic vesicles at day 18 or number of embryos at days 25 and 45 of pregnancy, in relation to OR

Table 4: LSM±SE of measured traits (BWD, BCSD, BFD, BMD, CRL and P_4) of nulliparous (P1) and parous (P2) ewes

Parameters	P1	P2
BWD (kg)	-0.93±0.21 ^b	0.47±0.15 ^a
BCSD (unit)	-0.04±0.03 ^b	0.06±0.02 ^a
BFD (mm)	-0.02±0.03 ^b	0.05±0.02 ^a
BMD (mm)	0.00±0.03	0.00±0.02
CRL25 (mm)	1.34±0.03	1.31±0.02
CRL45 (mm)	3.87±0.05	3.92±0.04
P_4 7 (ng mL ⁻¹)	3.64±0.14	3.71±0.10
P_4 25 (ng mL ⁻¹)	3.78±0.14	3.69±0.10
P_4 45 (ng mL ⁻¹)	3.80±0.17	3.77±0.12

Columns with different LSM superscripts differ significantly from each other ($p < 0.05$), D: Refers to the difference between the measurements at mating and day 45 after mating, CRL was measured at days 25 and 45 of pregnancy, P_4 was measured at days 7, 25 and 45 of pregnancy, BWD: Body weight difference, BCSD: Body condition score difference, BFD: Backfat thickness difference, BMD: Back muscle depth difference, CRL: Crown-rump length and P_4 : Progesterone level, P1: Nulliparous and P2: Parous ewes

Post-mating nutritional treatment significantly affected all the studied traits (Table 5, Fig. 3) except progesterone (P_4) level at day 7 of pregnancy. Post-mating under nutrition led to significant reduction in the body weight of females by 2.38 kg, compared to acquiring extra 1.71 kg in weight of the females in the post-mating over nutrition group through the first 45 days of pregnancy. At the same trend, BCSD, BFD and BMD were significantly decreased in the under-nourished females (-0.18 unit, -0.16 and -0.17 mm, respectively) and significantly increased in the over-nourished ones (0.65 unit, 0.21 and 0.18 mm, respectively). CRL of embryos was higher in the over-nourished animals. The under-nourished ewes had significantly higher P_4 level at days 25 and 45 of pregnancy, while the over-nourished ones recorded lower estimates than the control ewes. A clear reduction in PR45 estimates were recorded in the under- (0.64) and over-nourished (0.71) females compared to the controls (0.96). A double-fold EW were recorded in the over-nourished ewes (0.39) and three-folds in the under-nourished ones (0.54) at day 45 of pregnancy in comparison with the medium-nourished ewes (0.18).

Table 5: LSM±SE of measured traits (BWD, BCSD, BFD, BMD, CRL and P₄) in ewes fed 0.7 (N1), 1 (N2) and 1.3 (N3) of the recommended post-mating feed needs during the first 45 days of pregnancy

Parameters	N1	N2	N3
BWD (kg)	-2.38±0.21 ^c	-0.01±0.22 ^b	1.71±0.21 ^a
BCSD (unit)	-0.18±0.03 ^c	-0.05±0.03 ^b	0.65±0.03 ^a
BFD (mm)	-0.16±0.03 ^c	0.02±0.03 ^b	0.21±0.03 ^a
BMD (mm)	-0.17±0.03 ^c	0.00±0.03 ^b	0.18±0.03 ^a
CRL25 (mm)	1.31±0.03 ^b	1.27±0.03 ^b	1.40±0.03 ^a
CRL45 (mm)	3.86±0.05 ^b	3.81±0.04 ^b	4.02±0.04 ^a
P ₄ 7 (ng mL ⁻¹)	3.80±0.16	3.70±0.14	3.53±0.16
P ₄ 25 (ng mL ⁻¹)	4.20±0.19 ^a	3.68±0.16 ^b	3.35±0.18 ^a
P ₄ 45 (ng mL ⁻¹)	4.58±0.19 ^a	3.66±0.16 ^b	3.12±0.18 ^a

Columns with different LSM superscripts differ significantly from each other (p<0.05), D: Refers to the difference between the measurements at mating and day 45 after mating, CRL was measured at days 25 and 45 of pregnancy, P₄ was measured at days 7, 25 and 45 of pregnancy, BWD: Body weight difference, BCSD: Body condition score difference, BFD: Backfat thickness difference, BMD: Back muscle depth difference, CRL: Crown-rump length and P₄: Progesterone level

DISCUSSION

The current research examined the effects of breed, parity and post-mating nutrition on embryo wastage in early pregnancy in two breeds of Egyptian sheep, Rahmani and Barki. Both of the two breeds had low prolificacy but Rahmani sheep previously recorded higher estimates than Barki for both ovulation rate (1.29-1.57 vs. 1.16-1.20; Gabr *et al.*, 1989; Abdel-Mageed and Abo El-Maaty, 2012) and litter size (1.12-1.48 vs. 1.00-1.16; Mokhtar *et al.*, 1991; Aboul-Naga *et al.*, 1992; Abdel-Mageed and Abo El-Maaty, 2012). According to the difference between the two breeds in these two parameters, compared to the other Egyptian sheep, Rahmani and Barki breeds were considered in the present study.

In the respect of body measurements through the first 45 days of the nutritional treatment, significant reductions were recorded in under-nourished ewes for body weight, body condition score and backfat thickness, while significant increase in these measurements were attained in the over-nourished group. Both Parr *et al.* (1986) and Munoz *et al.* (2009) previously supported these results.

Pregnancy rate was not affected, in the present study, by both breed of ewes or their parities. The non-significant difference that recorded between the Rahmani and Barki ewes for pregnancy rate has been previously confirmed (Aboul-Naga *et al.*, 1992; Abdel-Mageed, 2009; Abdel-Mageed and Abo El-Maaty, 2012). For the effect of parity on pregnancy rate, Bari *et al.* (2003) concluded that parity or age of recipient ewes did not affect fertilization rate or embryo survival after transfer which is consistent with the non-significant effect of parity on pregnancy rate in our study. Wallace *et al.* (2010) stated that, both maternal body mass index at conception and gestational intake have a profound influence on pregnancy outcome in young, putatively growing adolescent sheep and may have implications for the nutritional management of pregnant adolescent humans.

Nutrient restriction during the first half of pregnancy impaired foetal growth (Igwebuike, 2010), associated with postnatal metabolic and endocrine disorders (Gilbert *et al.*, 2005) and cardiovascular disorders (Hawkins *et al.*, 2000), reduced uterine blood flow and uteroplacental glucose uptake (Bell, 1984) leading to fetal hypoglycemia and hypoxemia. While overfeeding prior to and after mating decreased the yield and quality of embryos after insemination (O'Callaghan and Boland, 1999) and impaired fetal growth (Igwebuike, 2010). The data reviewed

here indicate that post-mating nutritional practice significantly affected pregnancy rates at 18, 25 and 45 days after mating. In the under-nourished ewes, clear reduction in pregnancy rate was observed at day 18 post mating (32%) compared to the controls (only 4%) or the over-nourished females (18%). In the over-nourished ewes, pregnancy rate was reduced between days 18 and 45 of pregnancy by extra 11%, may be related to the reduction in P_4 of this group that reduced from 3.53 ng mL^{-1} at day 7 to 3.12 ng mL^{-1} at day 45 of pregnancy. Parr (1992) concluded that, pregnancy rate in ewes fed a high ration (200% of maintenance requirements) was significantly reduced when compared with those of ewes fed maintenance requirements which in accordance with the results of the present study. On the other hand, other researchers found no effect for the post-mating nutrition on pregnancy rate in sheep (Parr *et al.*, 1986; Munoz *et al.*, 2009; Sosa *et al.*, 2009; Debus *et al.*, 2012).

Both breeds of sheep and their parities, in the present study, did not record significant effects on the embryo wastage through the first 45 days of gestation. On the contrary, Yotov (2012) found significant effect for parity on embryonic mortality of sheep. For the effect of post-mating nutrition through the first 45 days of pregnancy on embryo wastage, the control ewes lost 18% of their ova ($p < 0.05$), compared to the over- (39%) or the under-nourished ewes (54%). Squires (2003) conclude that, animals which received inadequate nutrition after mating had a higher incidence of embryo mortality than those received adequate nutrition that is in harmony with our results. Since undernutrition induced changes in the endometrial sensitivity to steroid hormones at early stages of pregnancy that could adversely alter uterine environment to the detriment of embryo survival (Abecia *et al.*, 2006). Also, it reduced the ability of the embryo to secrete interferon-tau (IFN- τ), with a consequent increase in the production of $\text{PGF}_{2\alpha}$ from the endometrium which can initiate luteolysis (Gordon, 2004). On the other hand, the over nutrition seemed to adversely affect embryo survival. Parr *et al.* (1987) found 25-30% reduction in embryo survival arising from high-plane feeding. It would appear that high food intakes, via their stimulatory effects on both hepatic blood flow and the metabolic clearance rate of progesterone (Parr *et al.*, 1993), caused a decrease in plasma progesterone to levels that may compromise embryo growth and survival (Parr *et al.*, 1987; Robinson, 1990).

Amer (2010) concluded that, B-mode real-time ultrasonography is recommended as a reliable mean for early detection of gestation as early as 19-27 days after mating for measuring crown-rump length of embryos. Our results revealed that, the crown-rump lengths of embryos were higher ($p < 0.05$) in the over-nourished ewes that accord with results of Parr *et al.* (1986), Munoz *et al.* (2009) and Igwebuikwe (2010). On the other hand, Rhind *et al.* (1989a) found no difference in fetal size at days 21-26 between ewes receiving 50 and 150% of their maintenance needs.

Establishment and maintenance of pregnancy results from signaling by the conceptus and requires P_4 (Spencer *et al.*, 2007). The P_4 prepares the lining of the uterus for implanting of the ovum (Squires, 2003), stimulates blastocyst growth and elongation to a filamentous conceptus (Spencer *et al.*, 2007). In the context of measuring P_4 level in our study, the results indicated difference between the medium-nourished group and each of the under (+14.1%) and the over-nourished (-9%) groups at day 25 of pregnancy, increased at day 45 of pregnancy to be +25.1% and -14.8%, in the two groups, respectively. This inverse relationship between the level of post-mating nutrition and P_4 concentration was previously confirmed and may be attributed to the differences in clearance rate of progesterone (Parr *et al.*, 1987, 1993). The relationship among post-mating nutrition, embryonic survival and P_4 level was previously studied (Rhind *et al.*, 1989b;

Robinson *et al.*, 2002; Abecia *et al.*, 2006; Diskin and Morris, 2008). Diskin and Morris (2008) concluded that the systemic concentrations of progesterone preceding and following insemination affect embryo survival rate with evidence that too high or indeed too low concentrations were negatively associated with survival rate. Since the state of undernutrition induced changes in the endometrial sensitivity to steroid hormones at early stages of pregnancy that could adversely alter uterine environment to the detriment of embryo survival (Abecia *et al.*, 2006). The lower rate of embryo survival in the underfed ewes may have been associated with the more rapid increase of progesterone concentrations as concluded by Rhind *et al.* (1989b). On the other hand, Robinson *et al.* (2002) concluded that high feeding post-mating intake decreased pregnancy rate by reducing blood progesterone to concentrations that compromise embryo survival, that is consistent with our results. However, the previous reports did not clearly give evidences about the relationship between post-mating nutrition and embryonic death of sheep is related to the treatment of nutrition itself or to the acute changes in body weights of animals through the experimental periods. As a conclusion for this study, it is important to control the feeding allowance of ewes in early pregnancy to be around the maintenance requirements to get a considerable proportion of viable embryos.

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