Al-Muthanna J. For Agric Sci Online ISSN:2572-5149
Print ISSN: 2226-4086
Vol. 12 , Issue 2. 2025

https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/12.2/14

Awassi lambs' mortality Relationship with Dam age and Forkhead box O3 gene polymorphism

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Abstract:

The study was conducted on 78 Awassi ewes of three ages (2-3, 3- 4, and > 4 years) reared in private farms in central Iraq (Baghdad, Abu Ghraib District) throughout 2018 to investigate the effect of dam age and FOXO3 gene polymorphism (PCR-SSCP, exon 2) on lamb mortality before and after weaning. The results of the PCR product showed that the studied region included a single nucleotide polymorphism, and three genotypes were identified (AA, AC, and CC). The allele frequencies were 0.58 and 0.42 for A and C, respectively, and the genotype ratios were 39.75, 35.89, and 24.36% for AA, AC, and CC, respectively. Results showed a significant effect ($P<0.05$) of dam age on lamb mortality before weaning age, ewes with 2-3 years old were the highest lamb mortality (7.67%), and after weaning age, ewes with 2-3 years old were the highest lamb mortality (5.08%). FOXO3 genotypes affected the mortality rate significantly ($P<0.05$) from birth to weaning. The highest lamb mortality before the weaning rate was recorded in ewes with wild genotype (AA) and aged 2-3 years old, while the lowest mortality rate was noticed in the ewes group with mutant genotype (CC) and aged more than four years old, namely 7.65 and 3.32% respectively, and after weaning, namely 6.60 and 1.36% respectively.

Keywords: Awassi, Forkhead box O3, Dam age, Lamb mortality.

Introduction:

Awassi ewes are the most numerous sheep breed reared and widespread in the Middle East countries. The breed is acceptable as a multipurpose breed and contributes about 60% of Iraqi sheep (Al-Khuzai & Al-Wazeer, 2011). Longevity is crucial for breeding ewe flocks' economic success, reducing culling rates and female replacement costs for sheep farmers across Europe and beyond (McLaren *et al.*, 2020). One of the major problems that causes a high economic loss in sheep is lamb mortality under farming conditions. Many studies reported that the lamb's mortality is affected by various factors such as less care, the season of birth, berth type, birth weight, and breed (Dwyer & Lawrence, 2005; Sušić *et al.*, 2005; Khan *et al.*, 2006; Steinheim *et al.*, 2008; Mustafa *et al.*, 2014).

Dam age is a crucial factor that plays an important role in lambs' mortality from birth to weaning in many sheep breeds (Chniter *et al.*, 2009). Lambs' mortality is also affected significantly by many genes or genetic factors in the sheep genome. There are many genes strongly related to mortality rate, and many studies have referred to the effect of genetic factors on the livability of lambs (Raoof, 2018). One of these genes is the Forkhead box O3 gene (also known as FOXO3), which is considered a transcription factor and regulates hormonal, nutrient, and stress responses and affects cell survival and metabolism. The FOXO3 is an efficient factor for controlling cell functions, and degradation of sub-cellular localization affects its degradation, DNA-binding ability, and transcriptional activity (Boccitto & G Kalb, 2011).

The major aim of the current study is to investigate the relationship between dam age and FOXO3 gene polymorphism with lamb mortality from birth to weaning and appointment the results to improve the local sheep breed performance in Iraq, which suffers from many problems under farming conditions.

Materials and methods:

The current study was conducted on 78 Awassi ewes of three ages reared in private farms in central Iraq (Baghdad, Abu Ghraib District) through the year 2018. Blood samples were collected from the jugular vein using a disposable syringe and commercial tubes. DNA isolation was made according to the method of (Sambrook & Russell, 2001). PCR- SSCP of the fragment within exon 2 of FOXO3, and a specific primer was used for this purpose as below:

F-AACGCCAGCACAGTCAGC and R-CTTGTTCTCTTGGATGGTCT, primer annealing temperature was 64 C° (Byun, 2012).

Statistical analysis:

Data were analyzed using (SAS, 2012) computer program by the general linear model procedure (GLM) according to the following model:

Yijk = μ + Ai + Bj + (AB)ij + eijk

Where:

μ: the overall mean

Ai: effect of dam age (2-3, 3- 4, > 4 years).

Bj: effect of FOXO3 gene polymorphism (exon 2).

(AB)ij: interaction among ages and polymorphism.

eijk: is a random error.

Estimation of genotype frequencies: The genotypes were assigned based on restriction digestion patterns of the PCR products, and the allele and genotype frequencies were calculated by the standard formula (Falconer & Mackay, 1996):

Genotype frequency $= \frac{\boldsymbol{number\ of\ individuals\ of\ particular\ genotype}}{\boldsymbol{total\ number\ of\ animals\ of\ all\ genotypes}}$

Gene frequency = $\frac{2D+H}{2N}$

Where:

D: number of animals homozygous for a particular allele

H: number of heterozygous animals

N: total number of animals

The chi-square test was used to determine the significant differences among phenotypes:

$$X^2 = \sum \frac{(Observed\ No. - Expected\ No.)^2}{Expected\ No.}$$

Duncan's multiple range test (Duncan, 1955) was used to compare differences among means.

Results:

Results of the PCR product showed that the region that was studied (exon 2) included a single-nucleotide polymorphism (SNP), and three genotypes were noticed (AA, AC, and CC). Table 1) showed that non-significant differences in genotype distribution resulted from SNP, the allele frequencies were 0.58 and 0.42 for A and C, respectively, and the genotype ratio was 39.75, 35.89, and 24.36% for AA, AC, and CC, respectively.

Table 1. Distribution of the observed allele frequency for FOXO3 loci

Genotype	No.	%	χ^2	Allele frequency	
Intron 6					
AA	31	39.75	3.038	A	C
AC	28	35.89		0.58	0.42
CC	19	24.36			

Results showed a significant effect (P<0.05) of dam age on lambs' mortality before weaning age. Ewes 2-3 years old were the highest lamb mortality (7.67%) compared with the oldest ewes (more than 4 years old), which recorded the least mortality rate of lambs, namely 3.65% (Table 2).

Table 2. Combined effects of dam age and FOXO3 gene polymorphism on lambs' mortality before weaning

Dam age (year)	Mortality rate (%) ±S.D			Mean
	Genotypes			
	AA	AC	CC	
2-3	7.65± 0.88^{a}	5.32± 1.10^{abc}	7.30± 0.89^{ab}	6.75±0.79^{a}
3-4	6.66± 0.34^{ab}	4.67± 0.99^{abc}	7.32± 1.20^{ab}	6.21± 0.84^{a}
>4	3.65± 0.85^{bc}	3.34±0.90^{c}	3.32±0.82^{c}	3.43± 0.69^{b}
Mean	5.98±0.77^{a}	4.44±0.79^{a}	5.98± 0.68^{a}	5.47± 0.77
Numbers with different letters differ significantly (P<0.05).				

The results indicated that the lamb's mortality rates did not differ significantly according to the FOXO3 gene polymorphism, while the interaction between dam age and FOXO3 genotypes significantly affected (P<0.05) the mortality rate from birth to weaning. The highest lamb's mortality rate was recorded in ewes with wild genotype (AA) and aged 2-3 years old, while the lowest mortality rate was noticed in the ewes' group with mutant

genotype (CC) and aged more than four years old.

Results represented in Table 3 showed a significant effect (P<0.05) of dam age on lambs' mortality after weaning age. Ewes 2-3 years old had the highest lamb mortality rate (5.08%) compared with the oldest ewes (more than 4 years old), which recorded the least mortality rate of lambs, namely 1.90%. FOXO3 genotype polymorphism significantly affected lambs' mortality after weaning; the ewes with wild genotypes recorded the highest rate, namely, 4.32%, while the ewes with mutant genotype (CC) recorded the lowest mortality rate, namely, 2.79%.

Table 3. Combined effects of dam age and FOXO3 gene polymorphism on lambs' mortality after weaning

Dam age (year)	Mortality rate (%) ±S.D			Mean
	Genotypes			
	AA	AC	CC	
2-3	6.60± 0.57^{a}	4.63± 0.34^{b}	4.01± 0.07^{bc}	5.08±0.32^{a}
3-4	4.00± 0.29^{b}c	3.35± 0.31^{cd}	3.00± .01de	3.45± 0.20^{b}
>4	2.36± 0.36^{ef}	2.00±0.01^{fg}	1.36±0.32^{g}	1.90± 0.23^{c}
Mean	4.32±0.53^{a}	3.32±0.21^{b}	2.79± 0.18^{c}	3.47± 0.77
Numbers with different letters differ significantly (P<0.05).				

Results indicated a significant effect of interactions among genotypes and dam ages on the mortality rate of lambs after weaning, the highest rate was recorded in ewes with the wild genotype and 2-3 years old, compared with the lowest rate, which was noticed in the lambs that came from dams with mutant genotype and more than four years age.

Discussion:

Lamb mortality is one of the serious problems in sheep breeding, and the results of these studies follow most of the past studies in this scientific field. Bangar et.al. (2016) pointed to the significant effects of dam age on lamb mortality in sheep and referred to the fact that the dam age contributes to other factors such as breed or feeding system. Notter and Brown (2015) reported that lamb mortality is a complicated problem in the sheep industry and referred

to the importance of dam age, which interacts with various factors that affect this economic trait (Notter & Brown, 2015; Bangar *et al.*, 2016).

Other studies referred to the indirect effect of dam age on lamb livability through the correlation between dam age and lamb weight, which reflects on lamb mortality (AKTAŞ & DOĞAN, 2014; Elia, 2018). The current results are harmonic with many past studies that focused on the role of dam genetic factors in lamb mortality directly or indirectly. Mellado et.al. (2016) have referred to the indirect effect of dam genetics on lamb mortality through its influence on litter size, which is significantly related to lamb survival, especially during the pre-weaning period. Ehrhardt et al. (2020) reported that hybrid vigor, or heterosis, is an efficient strategy to reduce lamb mortality by nearly 20% and highlighted the importance of dam age, health, and maternal behavior on lamb livability. Nowadays, most modern studies focus on determining the role of specific genes, which are considered regulatory genes involved in newborns and associated with the incidence of newborn mortality.

Conclusion:

The results of this study suggest that the FOXO3 gene is one of the candidate genes contributing to the genetic variance of lamb mortality. This gene's polymorphism, which results from a single-nucleotide polymorphism, is considered a good tool for indirect selection to reduce lamb loss, interacting with many environmental or fixed factors. However, more studies are still needed to prove these results.

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