Ovine Mitochondrial DNA Polymorphism and Its Physiological Implications

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Introduction

Ovine mitochondrial DNA (mtDNA), similar to other mammalian mtDNA, contains 13 genes encoding polypeptides involved in oxidative phosphorylation, two ribosomal RNA genes, 22 transfer RNA (tRNA) genes and a D-loop region involved in the control of mitochondrial replication and transcription (Hiendleder *et al.* 1998). Polymorphism in ovine mtDNA has been investigated mainly in the highly variable D-loop and *cytochrome B* regions, resulting in the identification of five distinct mitochondrial haplogroups (Pedrosa et al. 2005; Meadows *et al,* 2007). While no information is available on ovine mitochondrial tRNA polymorphism, studying human mtDNA polymorphism resulted in the characterization of many disease-correlated mutations, most of them associated with mutations in tRNA genes (McFarland *et al.* 2004; Putz *et al.* 2007; Montoya *et al.* 2009).
Formation of the Assaf dairy sheep breed initiated with crossing of East Frisian rams with Improved Awassi ewes (Gootwine and Goot 1996). The Afec-Assaf strain was developed by crossing Booroola Merino rams homozygous for the B allele of the FecB locus with Assaf ewes (Gootwine *et al.* 2008). The aims of the present study, carried out in Afec-Assaf sheep, were to study the mitochondrial haplogroup profile of that strain, to gain insight into ovine mitochondrial tRNA polymorphisms and to study the association between mitochondrial polymorphism and productive and reproductive traits in sheep.

Material and methods

Animals and haplogroup identification. Maternal lineages were sorted for all 398 ewes present on 1 Jan 2010 in the Volcani Center Afec-Assaf flock book (Gootwine *et al.* 2008). DNA was prepared from whole blood of representative individuals from each maternal lineage and fragments encompassing part of the D-loop control region and part of the *cytochrome B* region were sequenced. The genotyped ewes and their relatives at the maternal lineages were affiliated to haplogroups based on the sequence criteria presented in Table 1.

tRNA sequencing. mtDNA fragments containing the 22 tRNA genes (Hiendleder *et al.* 1998) of five ewes from different maternal lineages from each of the A, B and C haplogroups were sequenced.

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Table 1: Sequence differences between haplogroups A, B, C and E according to Pedrosa *et al.* (2005) and Meadows *et al.* (2007) where, altogether, 36, 72, 25 and 3 sequences were assigned to haplogroups A, B, C and E, respectively

	Cytochrome B							D-loop					
Position[1]	14436	14467	14515	14636	14663	14845	14971	16069	16615	16622	16629	16701	16756
Sequence[1]	T	C	T	A	G	T	A	C	C	T	C	C	C
Haplogroup													
A	*	T	*	*	A	*	*	T	*	*	T	T	*
B	*	*	*	*	*	*	*	*	*	*	*	*	*
C	C	T	C	G	A	C	G	T	T	*	T	T	T
E	C	T	C	G	A	C	G	T	T	C	T	T	T

*Similarity to the published sequence.
[1]Accession no. AF010406.1 (Hiendleder *et al.* 1998).

Statistical analysis. Effects of mitochondrial haplogroup on prolificacy, lamb survival at birth and ewe longevity (birth to culling) was investigated by the General Linear Model procedure of the Jump In® computer package (SAS 2001). The data set included 1081 ewes with lambing records. The model for analyzing ewe longevity included the effects of: ewe genotype at the *FecB* locus (*BB*, *B*+, ++), ewe mitochondrial haplogroup (A, B, C), litter size of origin (1 to 4, and 5 and above) and sire within *FecB* group. The model for analysis of litter size included the effects of: ewe *FecB* genotype, parity no. (1 to 9 and 10 and above), mitochondrial haplogroup, ewes' sire within *FecB* group and first-order interactions. A similar model that also included litter size at birth was applied for lamb survival rate.

Results and discussion

Mitochondrial haplogroups in the Afec-Assaf. The 398 ewes presented in the Volcani Center Afec-Assaf flock were assigned to 35 maternal lineages comprising 1225 ewes, 1081 of them having, altogether, 4574 lambing records. Based on criteria presented in Table 1, the ewes were assigned to the A (n = 456), B (n = 473) and C (n = 154) mitochondrial haplogroups. A similar haplogroup profile, with the exception of the presence of the rare E haplogroup, has been described for the Improved Awassi (Meadows *et al.* 2007), which is the maternal breed parent of the Assaf and the Afec-Assaf. It is worth noting that only type B haplogroup mtDNA was observed in the Spanish Assaf (Pedrosa *et al.* 2007), reflecting the way in which this breed was formed - by upgrading crossing of

Assaf rams obtained from Israel with local Iberian breeds, where haplogroup B is the most frequent.

tRNA polymorphism. Sequencing the 22 tRNA genes of mtDNA of five unrelated ewes from each of the A, B and C haplogroups revealed that 17 of the tRNA genes were not polymorphic, and their sequences matched the published sequence (Hiendleder *et al.* 1998). The other five tRNAs—*tRNA-Val*, *tRNA-Lys*, *tRNA-His*, *tRNA-Ser* and *tRNA-Leu* (Table 2), were either polymorphic or deviated in their sequence from the corresponding published sequence.

Table 2: tRNA polymorphism in the ovine mtDNA of Afec-Assaf ewes

Gene	*tRNA-Val*	*tRNA-Lys*				*tRNA-His*		*tRNA-Ser*		*tRNA-Leu*
Position	1028	7719	7755	7759	7773	11605	11606	11649	11668	11710
Sequence[1]	A	T	A	T	G	T	C	T	G	C
A	A/G	G	*	*	*	*	T	*	A	del
B	*	*	*	T/C	*	*	*	T/C	A	del
C	*	*	G	*	G	T/C	*	*	A	del

[1]Accession no. AF010406.1 (Hiendleder *et al*. 1998).
*Similarity to the published sequence.

None of the tRNA polymorphisms were found across haplogroups, suggesting that the tRNA mutation occurred after the haplogroup ancestors diverged during evolution. Some tRNA polymorphisms (positions 7719, 7755, 7773 and 11606) were haplogroup-specific, suggesting a relatively early mutation event; other tRNA polymorphisms (positions 1028, 7759, 11605 and 11649) were identified within haplogroups, indicating relatively more advancedmutation events.

tRNA-Ser and *tRNA-Leu* sequences of all animals from the three haplogroups deviated at positions 11668 and 11710, respectively, from the published sequence (Hiendleder *et al*. 1998). This may reflect sequencing errors in the published sequence. Indeed, other published ovine mitochondrial sequences (Burgstaller *et al*. 2007) are in agreement with our observation.

The physiological impact of carrying mitochondrial tRNA mutations has been investigated intensively in humans. Some of these mutations have been found to be associated with pathologies (Putz *et al*. 2007). Interestingly, based on similarities in the 2D cloverleaf structure (Putz *et al*. 2007), two of the ovine *tRNA-Lys* mutations, namely T7719G and A7755G, found in the A and C haplotypes, respectively (Table 2), correspond to the A8308G and A8344G pathology-associated mutations, respectively, in human lysine mitochondrial tRNA. The C11606 T mutation, located at the 3' end of the TΨC loop of the *tRNA-His*, corresponds to the human *tRNA-His* G12192A pathology–associated mutation (Shin *et al*. 2000).

Association between mtDNA polymorphism and production traits. mtDNA haplogroup had no significant effect ($P > 0.05$) on ewe longevity (averaged 1678 days) or on lamb survival rate (averaged 0.89) at birth. However, least-squares analysis showed a significant ($P < 0.0004$) effect of haplogroup on prolificacy, with an average prolificacy of 2.19, 2.29 and 2.41 lambs born/lambing for haplogroups A, B and C, respectively. As expected, in the same analysis, genotype at the *FecB* locus also significantly ($P < 0.0001$) affected prolificacy, with an average litter size of 1.64, 2.44 and 2.81 lambs born/lambing for ++, *B*+ and *BB* genotypes, respectively. Sire effect and parity effect were also significant ($P < 0.0001$) on prolificacy, while the mitochondrial x *FecB* interaction was not.

Further research is needed to elucidate the association between mitochondrial polymorphism and prolificacy in sheep. Nevertheless, it is suggested that this effect should be taken into consideration in planning crossbreeding in sheep when prolificacy is one of the breeding goals.

Conclusion

To date, ovine mtDNA polymorphism has been demonstrated mainly in the highly variable D-loop and *cytochrome B* regions. Studying the Afec-Assaf strain in which the A, B and C mitochondrial haplogroups were identified, we revealed tRNA polymorphisms: some of the mutations may resemble pathology-associated mutations in human mitochondrial tRNA. In addition, our results suggest that mtDNA polymorphism affects ewe prolificacy.

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