



# Veterinary and Comparative Biomedical Research

# CASE REPORT

# First Report of Idiopathic Advanced Follicular Atrophy of the Thyroid Gland in Three Miniature Donkeys

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#### Running Title

Follicular Thyroid Atrophy in Miniature Donkeys

#### **Abstract**

Thyroid gland disorders in equines, particularly in donkeys, are seldom reported in the veterinary literature. To the best of our knowledge, this is the first documented study focusing on pathological lesions of the thyroid in equines, especially miniature donkeys. The objective of this report is to provide insight into the histopathological, hormonal and electrolyte alterations observed in the thyroid glands of three animals. In this study, three miniature donkeys housed at the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran, were subjected to necropsy procedures for educational purposes. Prior to necropsy, blood samples were collected from each animal to assess serum thyroid hormone levels. Following euthanasia, the thyroid glands were carefully dissected and submitted for histopathological evaluation using standard microscopic techniques. Histopathological analysis revealed notable lesions within the thyroid tissue, including infiltration of adipose (fat) cells, increased deposition of connective tissue (fibrosis), and structural collapse of thyroid follicles. These morphological changes were accompanied by a marked reduction in serum concentrations of triiodothyronine (T3) and thyroxine (T4), as demonstrated by laboratory analysis of the collected blood samples. The findings of this report confirm the presence of advanced follicular atrophy in the thyroid glands of the examined miniature donkeys. This degenerative change appears to be directly associated with the observed decrease in circulating thyroid hormones, suggesting a state of hypothyroidism. These results underscore the need for further research into thyroid function and pathology in donkeys and other equids.

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#### Introduction

As a unique species of equine, the donkey has certain specific variations from the horse (1). Miniature donkeys are a pretty species of donkey (2). They are kept for work and milk production (3) and because of the attractiveness of these species, they are kept as pet animals (2).

The thyroid gland is responsible for the secretion of triiodothyronine (T3), thyroxine (T4) and plays an important role in the physiology and endocrinology of the body (1).

Information about thyroid gland diseases in equines especially miniature donkeys is scarce, and disorders in this organ are rare (4). Idiopathic follicular atrophy (IFA) is characterized by the loss of thyroid parenchyma, replacement by adipose connective tissue, and follicular collapse (5). Evidence suggests that this condition induces hypothyroidism, disrupting hormonal homeostasis and posing severe health risks, including life-threatening complications if the lesion persists. Although the exact pathogenesis remains poorly understood, heavy metal toxicity and autoimmune mechanisms are considered potential contributing factors (6).

IFA has been reported in dogs (7), cattle (8) and horses (6) and here we report IFA in miniature donkeys for the first time in the veterinary literature.

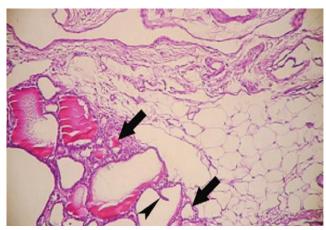
# **Materials and Methods**

Three miniature donkeys with a BCS of 1 (male, 4-5 years old) were necropsied at the Veterinary Faculty of Shahid Bahonar University of Kerman, Iran, for educational purposes based on ethical consideration. They were not cohoused and were not related to each other. The temperature, heart and respiratory rates were normal and they did not show any clinical signs, except some poor condition. These animals were clinically healthy and showed no signs of disease. Before the necropsy, blood samples were collected from the jugular vein using sterile tubes without anticoagulants for serum biochemical analysis and tubes with anticoagulants for hematological analysis. The collection site was disinfected with alcohol prior to sampling, and all precautions were taken to minimize stress to the animals. For serum preparation, blood samples were kept at room temperature for 30 minutes to allow clotting. Subsequently, the samples were centrifuged at 3000 rpm for 10 minutes using a Hettich Universal 320 centrifuge (Andreas Hettich GmbH & Co. KG, Germany), and the serum was aliquoted into sterile microtubes and stored at -20°C until analysis. ELISA kits were used to measure T4 (Monobind. Inc, made in the USA) and T3 (Pishtaz Teb, Tehran, Iran). It was not possible to measure diet and serum iodine levels.

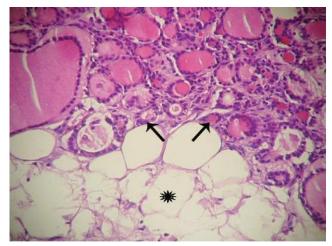
Complete tissue samples were collected and fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, cut at 5  $\mu$ m, and stained with hematoxylin-eosin (H&E), for histopathological studies.

#### Results

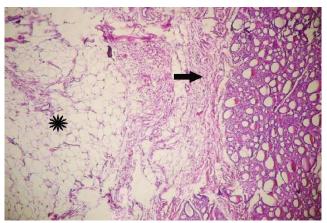
The results of hematology and serum biochemical analysis are presented in Table 1, but did not show significant changes, except serum concentrations of  $T_3$  and  $T_4$  which decreased significantly. Serum concentrations of  $T_3$  and  $T_4$  in necropsied miniature donkeys are presented in Table 2. Normal values were defined by reference (9).



**Figure 1.** Adipose tissue is seen next to clusters of follicles in irregular shape and varying size. The follicles are shrunken with empty and/or mild amount colloid (arrows). Papillary projections (arrowhead) have extended into the follicular space (×100, H&E)



**Figure 2.** Mature fat cells (asterisk) are in vicinity of degenerated and atrophic follicles (arrows). The mild increase of connective tissue in the interstitium of glands is visible (×200, H&E)



**Figure 3.** A part of the thyroid gland is replaced by fat cells (asterisk) and connective tissue (arrow). The most remained thyroid follicles have small size with little eosinophilic colloid  $(\times 40, H\&E)$ 

**Table 1.** Hematobiochemical analysis of the three miniature donkeys

Necropsy of complete tissue samples did not show any significant changes. Histopathological evaluations showed changes in the thyroid glands confirming advanced follicular atrophy. Adipose tissue is seen next to clusters of follicles in irregular shape and varying size. The follicles are shrunken with empty and/or a mild amount of colloid (arrows). Papillary projections (arrowhead) have extended into the follicular space (Figure 1). Mature fat cells (asterisk) are in the vicinity of degenerated and atrophic follicles (arrows). A mild increase in connective tissue in the interstitium of glands is visible (Figure 2). A part of the thyroid gland is replaced by fat cells (asterisk) and connective tissue (arrow). The remaining thyroid follicles are small size with little eosinophilic colloid (Figure 3).

Cases	RBC (× 10 <sup>6</sup> /μL)	PCV (%)	WBC (× 10³/μL)	TPP (g/l)	Ca <sup>+2</sup> (mmol/L)	Phosphorus (mmol/L)	Na + (mmol/L)	K + (mmol/L)	GGT (IU/L)
1	5.8	36	11	72.1	2.96	0.72	125	4.22	121
2	5.9	35	10.5	74.2	2.94	0.95	130	4.25	132
3	5.7	34	11.2	73.5	3.08	0.85	132	4.21	115

RBC: Red Blood Cell (Normal ranges:4.4 - 7.1), PCV: Packed Cell Volume (Normal ranges:27 - 4.2), WBC: White Blood Cell (Normal ranges:6.2 - 15), Ca +2: Calcium (Normal ranges:2.2 - 3.4), Na +: Sodium (Normal ranges:125 - 138), K +: Potassium (Normal ranges:3.2 - 5.1), GGT: Gammaglutamyl-transferase (Normal ranges:21 - 177), TPP: Total Plasma Protein (Normal ranges: 58 - 76)

**Table 2.** Serum concentration of  $T_3$  and  $T_4$  in the three necropsied miniature donkeys

Parameters	Necropsied Donkeys*	Normal Value* (11)
T <sub>3</sub> (ng/dl)	$1.85 \pm 1.14$	18.13±1.38
$T_4 (\mu g/dl)$	$48.16 \pm 27.61$	82.97±7.19

<sup>\*</sup> Mean ± SD

# Discussion

IFA is an infrequent pathological condition in veterinary literature, with limited documentation regarding its clinical presentation and pathophysiology (5-10). This rarity underscores the importance of detailed histopathological and clinical observations in the current case to enhance our understanding of the disease.

Histologically, IFA is characterized by progressive atrophy of the follicular epithelium, with subsequent replacement by adipose tissue and a minimal inflammatory response. This indicates a likely chronic degenerative process, with minimal evidence to support an acute or immune-mediated etiology. The atrophied gland appears smaller and lighter in color than normal, a hallmark finding in cases of advanced hypothyroidism (5). This chronicity aligns with the clinical course of hypothyroidism in dogs,

often presenting subtly before progressing to overt hormonal imbalances.

In its early stages, IFA manifests as focal atrophy, often confined to specific regions of the thyroid gland. Lesions in these early cases are typically associated with mild clinical signs of hypothyroidism, as previously described (7-8). Microscopically, affected areas exhibit small follicles lined by tall columnar epithelial cells, with reduced colloid content, contrasting with the surrounding normal thyroid tissue. This focal pattern might reflect an uneven progression of the atrophic process or localized susceptibility within the thyroid gland.

The advanced form, as observed in the present cases, demonstrates extensive pathological changes. The thyroid gland is predominantly replaced by adipose tissue interspersed with occasional clusters of small follicles, often containing vacuolated or clumped colloids (5-7). The prominence of adipose tissue may imply a long-standing degenerative process, potentially exacerbated by metabolic imbalances.

Advanced IFA has been consistently linked to clinical hypothyroidism in dogs, as evidenced by reduced circulating concentrations of thyroid hormones (5-7). The imbalance in thyroid hormones (T3 and T4) reflects the functional impairment caused by follicular loss and atrophy. It was not possible to measure diet and serum iodine levels.

In this report, decreased levels of  $T_3$  and  $T_4$  confirm hypothyroidism, corroborating the histopathological findings.

While hypothyroidism is recognized as a significant clinical condition in dogs, its prevalence and clinical importance in other species remain limited (11). This highlights the species-specific nature of thyroid gland pathology, where certain breeds or genetic predispositions in dogs may contribute to the higher incidence of hypothyroidism and its pathological correlates, such as IFA.

# Conclusion

Here we reported an IFA of the thyroid gland in three miniature donkeys for the first time in the veterinary literature, which could lead to hypothyroidism. Base on the results of this report hypothyroidism resulting from IFA has no clear clinical signs, so it is advised to diagnose hypothyroidism, with thyroid hormone measurement.

# Acknowledgements

Not applicable.

#### **Author Contributions**

Ali Asghar Mozaffari: designed the report and wrote the manuscript, Amin Derakhshanfar: performed histopathological evaluations, Omid Mohammadpour: helped in writing manuscript, Maryam Hadadipour Zarandi: prepared hematological and histopathological samples.

# Data availability

All data generated or analyzed during this study are included in this published article.

#### **Ethical Approval**

All procedures involving the miniature donkeys were performed with informed consent from the animal owners, and veterinary care was provided to ensure animal welfare throughout the study. No invasive procedures were performed solely for research purposes; samples and data were collected as part of routine clinical investigations or post-mortem examinations. The research complies with relevant legislation and ethical guidelines governing veterinary clinical research.

#### **Conflict of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

#### Consent for Publication

Not applicable.

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