Morphohistology data of normal variation of the thyroid glands from tadpoles in Amphibian Metamorphosis Assays using automated image analysis by machined learned algorithms.
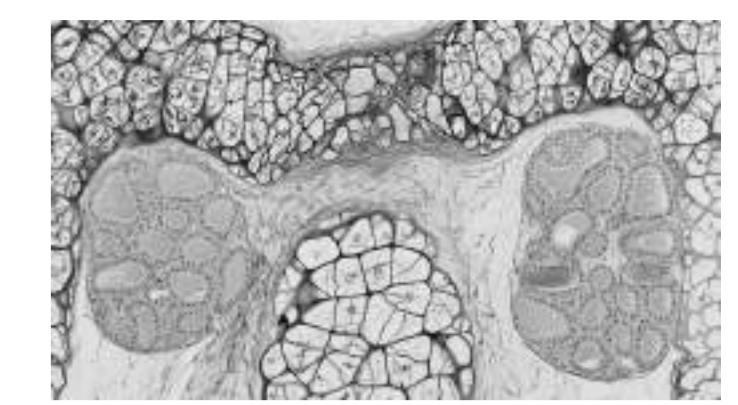
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Introduction

The histological evaluation of thyroid glands is one endpoint for the Amphibian Metamorphosis Assay using the African Clawed Frogs (*Xenopus laevis*). Thyroids are evaluated for thyroid atrophy/hypertrophy, follicular cell atrophy/hypertrophy, follicular cell hyperplasia, and reduced/increased follicular area (Grim et al., 2009; OECD, 2007). Knowledge of the morphological variation of normal thyroid glands in the corresponding developmental stage of tadpoles is a prerequisite for the assessment of possible induced endocrine effects by the toxicological pathologist. Heterogenicity among thyroid glands from control animals, may be associated with larval stage, breeder, facilities, alimentary conditions, possible solvent effects, etc. In addition, there are differences between the various stages, which can complicate investigation and interpretation. To avoid bias, the application of image analysis is an important tool.

Material and Methods

Histological sections of thyroid glands stained with haematoxylin and eosin from control tadpoles of 12 studies, n = 301 tadpoles at stages 57 to 60 (Nieuwkoop and Faber, 1994) from three test facilities (CRO A, CRO B, CRO C) were evaluated by pathologists and the corresponding whole slide images were analysed using an automated procedure in QuPath: Open source software for digital pathology image analysis (Bankhead et al., 2017). In addition, the high dose group from a study with test-item induced hypertrophy was analysed using the machine learned algorithms to evaluate the use of this tool to support traditional histologic evaluation. The data obtained include:

- total thyroid gland area
- ratio between the follicular epithelium vs. total area
- height of the follicular epithelium
- total colloid area
- ratio between epithelium and colloid area
- number of epithelial cells.

Results and Discussion

The results provide a basis for understanding normal variation for specific larval stages, and data revealed differences in thyroid gland morphometry among test facilities. Furthermore, there were minor differences between controls in water and controls in solvents. Thus, thyroid findings need to be carefully interpreted and compared to study stage-matched controls for conclusive results.

The methodology was also applied to a study with induced thyroid gland hypertrophy and hyperplasia and hyperplasia. Automated image analysis showed marked and significant differences between the control group and high dose for all parameters. It is concluded that image analysis by automated machined learned algorithms enabled objective quantitative data for the thyroid gland and across the complete 2D histological section. Therefore, the image analysis is considered a valuable and accurate tool to support the microscopic evaluation by the pathologist.
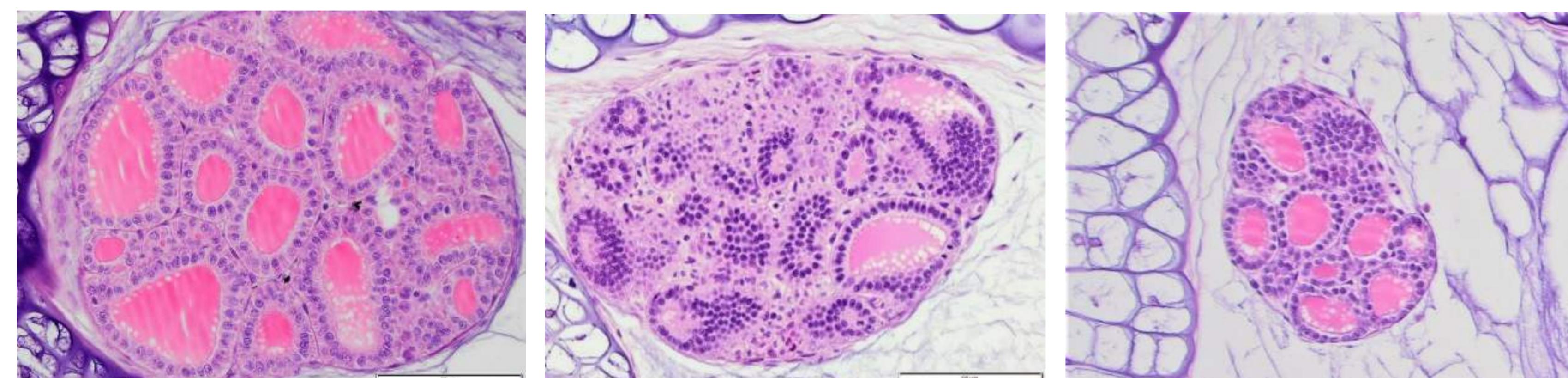
Thyroid. Stage 59. Normal.

Thyroid. Stage 59. Atrophy (Note reduced glandular size and reduced epithelial height and follicular area.

Thyroid. Stage 57. Atrophy (Note: bead-like appearance of epithelia. Reduced glandular size and reduced epithelial height and follicular area.
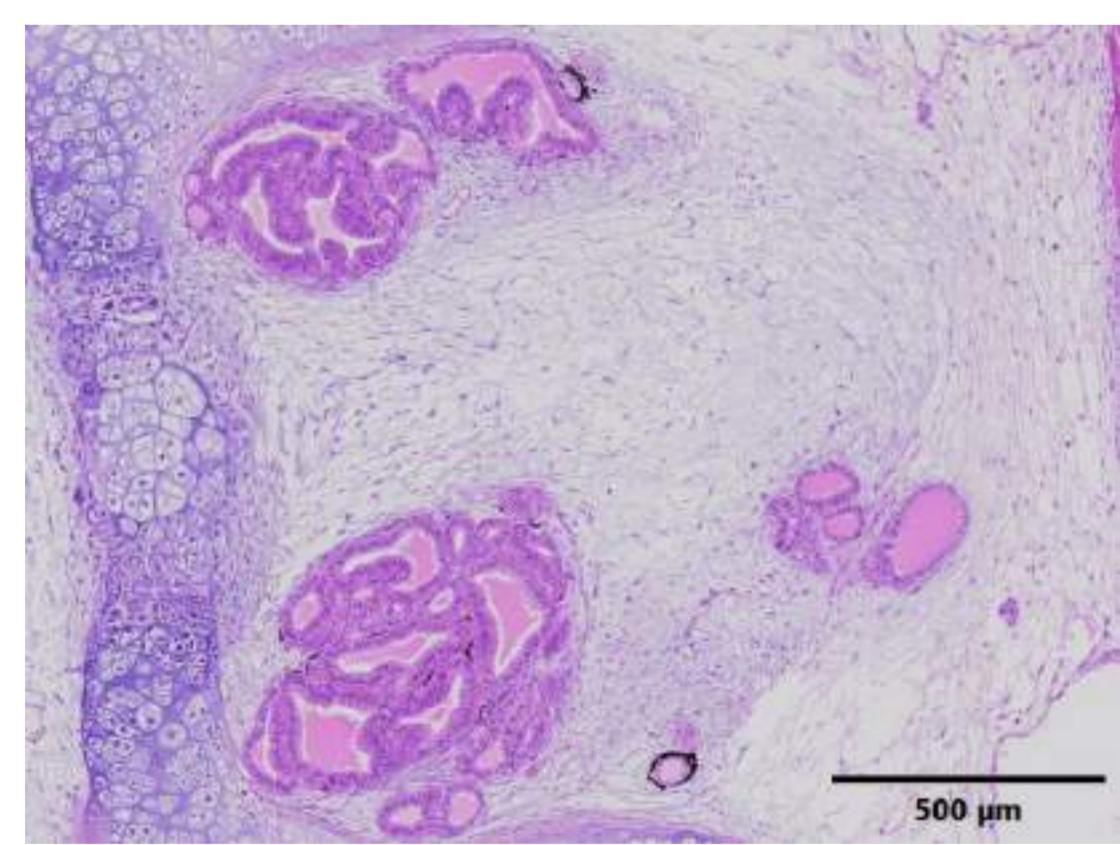
Thyroid. Stage 59. Follicular cell hyperplasia. Note shape of thyroid gland and infoldings of epithelium.
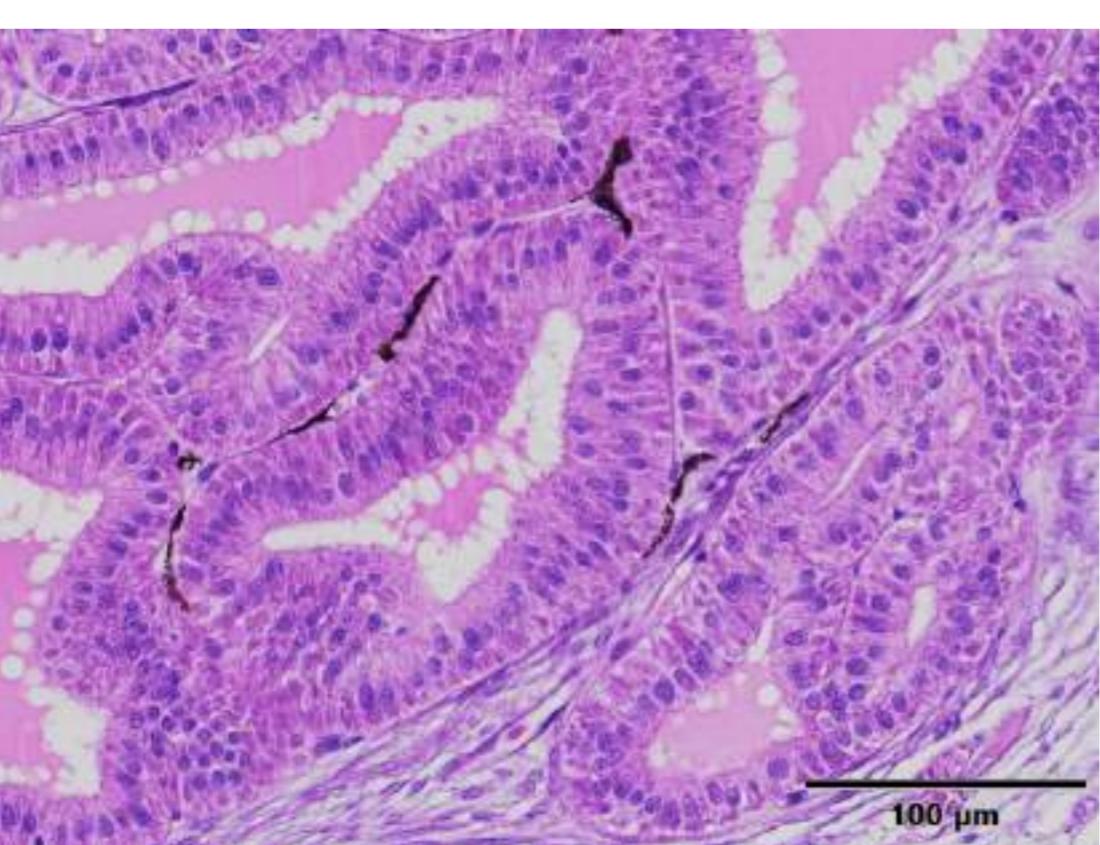
Thyroid. Stage 59. Follicular cell hyperplasia and hypertrophy.

Thyroid. Stage 59. Follicular cell hypertrophy. Note increased height of follicular cells and increased follicular area.
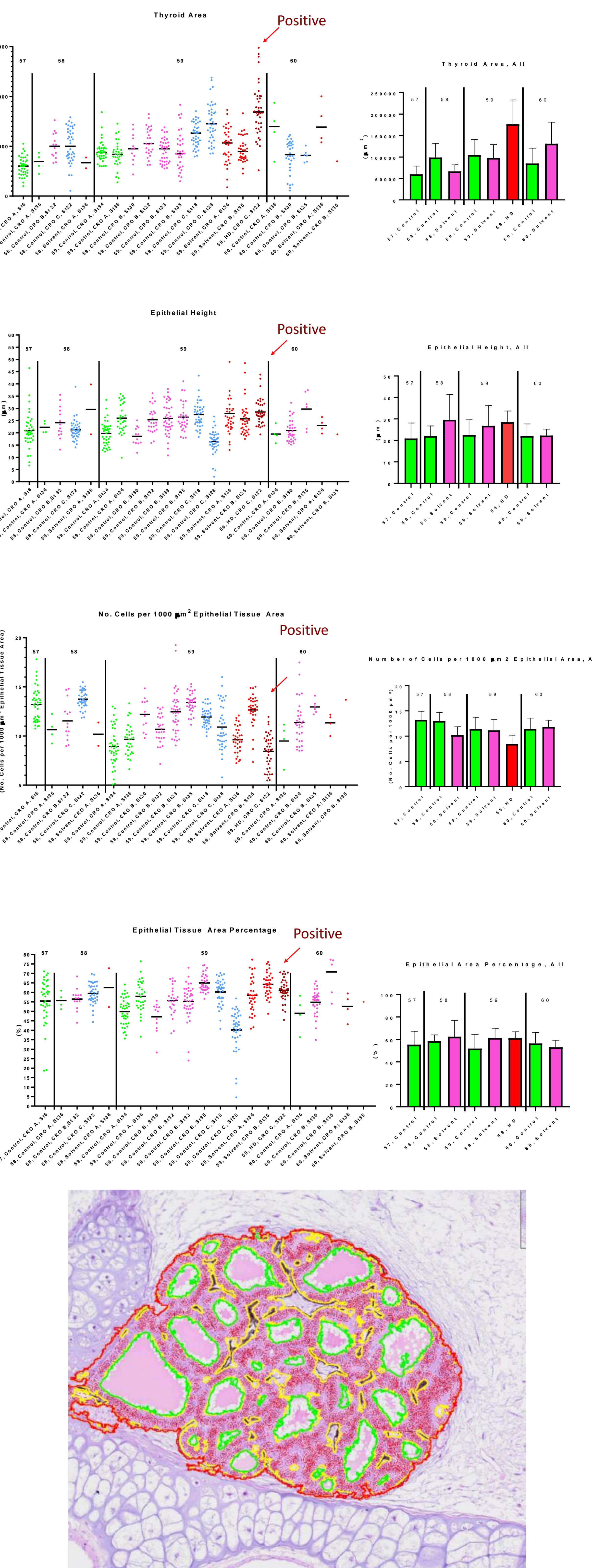
Thyroid. Result of Image Analysis

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