"The stray dogs as sentinel species for environmental risks in the Land of Fires (Campania, Italy)"

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In Campania Region, an area known as the 'Land of Fires', illegal dumping of toxic substances has made environmental contamination a major concern. From a One Health perspective, stray dogs represent an excellent sentinel species for human environmental risks. To this aim, fifty male stray dogs from the provinces of Naples and Caserta were enrolled, and testicular samples were processed for histological, chemical, and molecular analyses. Animals were classified into four groups, ranging from normal spermatogenesis to intratubular seminoma. Metal concentrations were assessed by inductively coupled plasma mass spectrometry. Collagen deposition was evaluated by Masson's trichrome and Picrosirius red staining. Steroidogenic enzymes (17β-HSD and P450 aromatase) were analysed by immunohistochemistry and Western blot, while testicular remodelling was investigated by immunofluorescence for vimentin and α-smooth muscle actin. Finally, oxidative status was examined by measuring hydrogen peroxide production and superoxide dismutase activity, while sperm chromatin condensation was assessed by Toluidine Blue staining using an automated whole-slide image analysis based on k-means clustering. Significant differences in cadmium, nickel, and uranium levels were observed across groups, with the highest burden in neoplastic testes. Testicular damage was associated with progressive stromal remodelling, increased collagen deposition, changes in collagen composition, and altered steroidogenic markers. In parallel, degenerated and neoplastic testes showed a clear redox imbalance, characterized by increased H_2O_2 production and reduced SOD activity, indicating impairment of the local antioxidant defence. These alterations were associated with cytoskeletal disorganization, as reflected by progressive loss and redistribution of vimentin and α-smooth muscle actin, and with functional sperm impairment, including reduced epididymal sperm concentration and defective chromatin condensation. These findings reinforce the value of the dog within the One Health framework, linking environmental contamination to reproductive dysfunction and supporting its role as a sentinel for environmental health monitoring