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Comet Assay validation of male germ cells isolated from seminiferous tubules

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The *in vivo* alkaline comet assay is a well-established method for detection of DNA damage in somatic cells, either as a follow-up testing of positive or equivocal in vitro test results or to evaluate local genotoxicity. Although it is widely used on somatic cells, there are only few studies available demonstrating its capability to detect mutagenicity in germ cells, which is more often required for an optimal risk assessment. Moreover, other alternative methods, assessing the induction of germ cell mutations, require very large numbers of animals and often lack practicality and efficiency. Therefore, an internal validation study was performed to verify the performance of the alkaline comet assay with male germ cells isolated from the seminiferous tubules.

For calculation of inter-analyst variability, three analysts independently prepared single cell suspensions of germ cells isolated from the seminiferous tubules, which were collected from male gonads. As freezing of the gonads led to cell damage and resulted in non-evaluable results, the cell suspensions were directly used to prepare comet slides. As the comet slides can be stored for a period of up to 3 months at room temperature under dry conditions and protected from light, they can be analyzed later without initiating a new animal study, depending on the results in somatic cells.

Within the validation study, experimental competency was successfully demonstrated, proving the ability to obtain single cell suspensions of sufficient quality for the performance of the alkaline comet assay. As required by the OECD TG 489, a positive response with three increasing concentrations of ethyl methane sulfonate (EMS) was noted, indicating that the positive control had reached the gonads and the observed DNA damage is concentration-related. Moreover, repeatability and inter-analyst variability were calculated and showed small standard deviations both within the dose group and between analysts.

In summary, the integration of analysis of germ cells isolated from the seminiferous tubules into the standard OECD TG 489 test protocol for somatic mutation offers an effective option for germ cell mutation testing and optimizes and reduces the use of animals while requiring less time and resources.

Keywords:
comet assay, male germ cells, Wistar rat, seminiferous tubules, OECD 489.