

A screenshot of the OPEN COMET software

The following comet parameters are generated on a spreadsheet by the software program.

Comet Area: Number of pixels in the comet

Comet Length: Length of the comet region in pixels

Comet DNA Content: Sum of pixel intensities inside the comet

Comet Average Intensity: Comet DNA content divided by comet size

Head Area: Number of pixels inside the head

Head Diameter: Length of the head in pixels

Head DNA Content: Sum of pixel intensities inside the head

Head Average Intensity: Head DNA content divided by head size

Head DNA %: Head DNA content as a percentage of Comet DNA content

Tail Area: Number of pixels in the tail

Tail Length: Length of the tail in pixels

Tail DNA Content: Sum of pixel intensities inside the tail

Tail Average: Tail DNA content divided by tail size

Tail DNA %: Tail DNA content as a percentage of Comet DNA content

Tail Moment: This is the product of the tail length and the fraction of total DNA in the tail

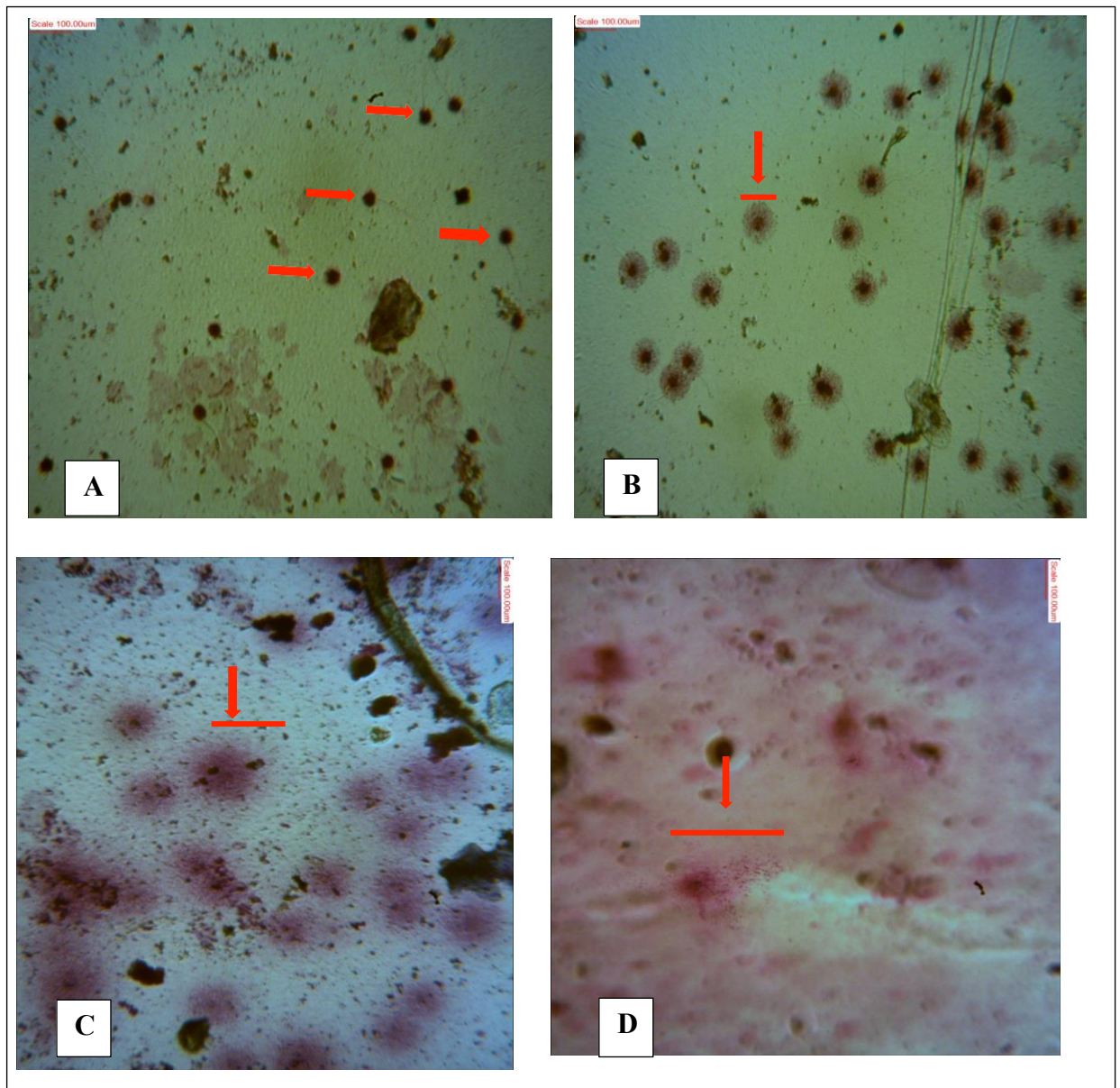
(Tail moment = tail length x % of DNA in the tail)

Olive Moment: This parameter represents the product of the percentage of total DNA in the tail and the distance between the centers of the mass of head and tail regions.

100 cells were analyzed per sample and the mean score of each sample was used as the representative score for that sample in further computations.

2.2. Statistical Analysis

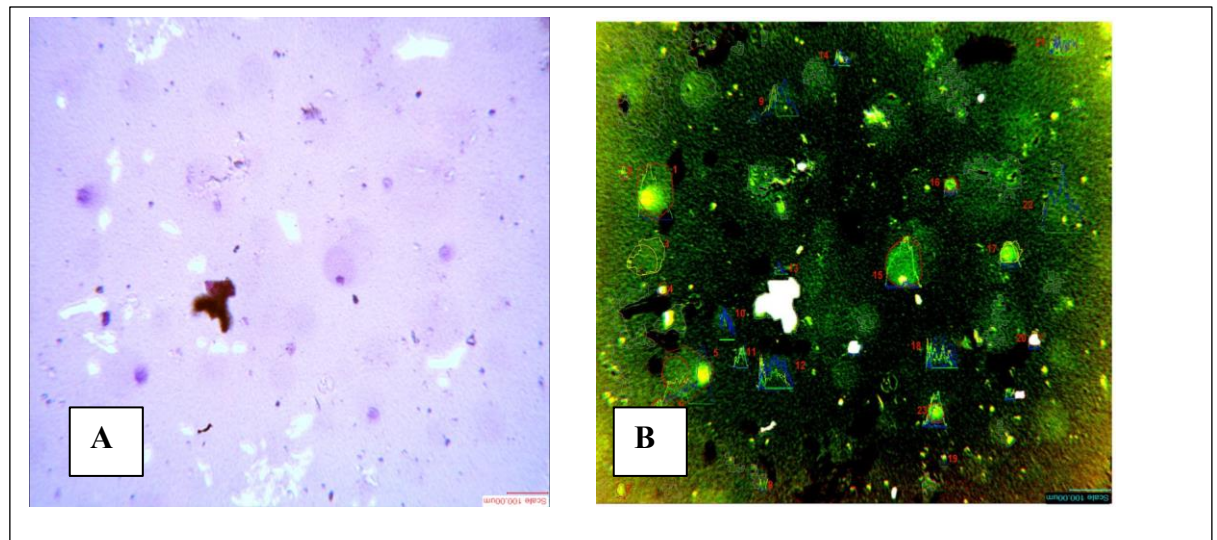
The data generated by OPEN COMET were subjected to analysis for descriptive statistics and correlation in SPSS statistical software, version 22.0.



Sperm DNA fragmentation was observed and scored in the study.

A. No fragmentation observed on the sperm head. **B.** Little fragmentation as seen by the dispersion of the DNA around the sperm heads. This halo becomes larger with increasing fragmentation as seen in the tail in C and D

Magnification: X400



Slide containing Comets captured as stained with Giemsa (**A**) and after colour inversion to permit comet scoring using the open comet software (**B**). Notice the numbers assigned to each comet by the software