The use of computed assisted semen analysis (CASA) as a method for environmental and toxicological risk assessment – The use of different chambers as sensitivity factor

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Reproduction is the biological process by which new individual organisms are produced. It is a fundamental feature of all known life; each individual organism exists as the result of reproduction. Spermatozoa production results in the daily formation of many millions of spermatozoa. The purpose of spermatogenesis is to establish and maintain daily output of fully differentiated spermatozoa that in mammals ranges from more than 200 million in man to 2-3 billion in bull.

Environmental pollution is increasing by rapid leaps worldwide due to the development of modern human society. After industrial revolution, a huge amount of toxic chemicals are disposed into the environment from various anthropogenic activities including industry, agriculture, mines, transportation and settlement. Stress to toxic metals is one of the best examples of evolution driven factors derived by anthropogenic activities. A rapid rate of metal pollution can be a strong selection force causing rapid evolutionary changes in organisms manifested as metal tolerance occurring over time scales as centuries and even decades. Conditions also develop so as to promote uneven distribution of essential elements in the animal organism and change their interaction.

The aim of this study is to describe CASA method useful for estimation of changes related to environmental biology and ecology. In relation to conference topics target of this study is related mainly to modelling (the use of different chambers) related to sensitivity.

Semen and spermatozoa analysis evaluates certain characteristics of semen and spermatozoa contained therein. It is done to help evaluate male fertility. Depending on the measurement method, just a various characteristics may be evaluated. The most common reasons for semen analysis are related to infertility investigation.

Motility is the basic parameter used for CASA analysis. Usually in each sample the following parameters were evaluated – percentage of motile spermatozoa (motility > 5 μm/s), percentage of progressive motile spermatozoa (motility > 20 μm/s), DCL (distance curved line; μm), DAP (distance average path, μm), DSL (distance straight line, μm), VCL (velocity curved line, μm/s), VAP (velocity average path, μm/s), VSL (velocity straight line, μm/s), ALH (amplitude of lateral head displacement, μm) and BCF (beat cross frequency, Hz). From these parameters also others are calculated as linearity – VSL:VCL, straightness – VSL:VAP and wobble – VAP:VCL.

In this study rabbit spermatozoa motility parameters, measured using different evaluation chambers, were compared. The measurement was done using CASA (Computer Assisted Semen Analysis) system; each sample was placed into four different chambers – microscopic slide, Zander Spermometer, Standard Count Analysis Chamber Leica 20 micron and Makler Counting Chamber. CASA showed that all measured parameters varied depending on chamber used as follows: an average spermatozoa concentration was 1.02 – 1.17 x 10^6/ml, the percentage of motile spermatozoa was in range 59.85 – 77.78% and spermatozoa with progressive motility was ranged from 46.14 to 68.57%. Of other parameters, DAP was 19.23 – 24.44 μm, DCL 37.43 – 47.20 μm, DSL 14.27 – 18.92 μm, VAP 45.26 – 57.31 μm/s, VCL 87.45 – 110.37 μm/s, VSL was 33.77 – 44.31 μm/s, straightness 0.71 – 0.76, linearity 0.36 – 0.40, wobble 0.50 – 0.52, ALH 4.18 – 4.60 μm and BCF 23.58 – 28.16. Statistical analysis detected significant differences in almost all studied parameters in regards to evaluation

chamber used. Particularly, highest values for concentration, percentage of motile and progressive motile spermatozoa were detected when microscopic slide with coverslip was used as a chamber. In parameters of the distance, velocity, linearity, straightness and BCF the highest values were obtained using Zander Spermometer, whilst the amplitude of lateral head displacement was the highest in the Makler chamber. These results clearly suggest that the type of evaluation chamber may influence a reliability of measurement of spermatozoa parameters.
Our previous in vitro experiments detected various significant dose and time dependent decrease of percentage of motile spermatozoa. Similar tendencies were observed for progressive spermatozoa motility. Detail motility analysis (curvilinear path, average path, straight motility path) show significant differences mainly after various time periods of culture. For further analysis we suggest to calculate concentration dependent decrease of spermatozoa progressive motility up to 50% of control (CDSM50) which should be calculated from at least five replicates using standard statistical tests and compared to progressive spermatozoa motility in control in various time periods.

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