

## Azoospermia: Virtual Reality or Possible to Quantify?

TG Cooper, B Hellenkemper, J Jonckheere, N Callewaert, AJ Grootenhuis, WM Kersemaekers, A Leung, C Wang. Institute of Reproductive Medicine of the University Clinic, Domagkstrasse, Germany.

Three sperm counting methods were compared within and between three centres to determine the sensitivity and reproducibility of assessing low sperm concentrations.

Two methods were performed using phase contrast microscopy, with and without centrifugation, and one method was performed using fluorescence microscopy (using the DNA stain Hoechst 33342) without centrifugation.

Semen samples were serially diluted in fluorescent dye-containing fixative and sperm concentrations were assessed in duplicate in the central field (100µl) of re-usable Neubauer chambers (phase contrast microscopy), in the whole field of disposable 25µl Leja chambers (fluorescence microscopy) and in wet preparations (up to 1950 microscopic fields) of the pellet obtained after centrifugation at 3,000g for 15 min (phase contrast microscopy).

Agreement between the three participating centers was good with lower limits of quantification (the concentrations for which counting errors (the standard error of the number of spermatozoa counted expressed as a percentage of the count) are  $\leq 20\%$ ) were determined to be 150,000/ml for the Neubauer chamber (phase contrast microscopy) and 500/ml for the Leja chamber (fluorescence microscopy).

These are equivalent to 300,000/ml and 1,000/ml for undiluted semen.

The centrifugation method consistently, seriously and significantly underestimated mean sperm concentration compared with the other methods by an average of 49%.

In conclusion, the accurate measurement of low sperm counts is facilitated by the use of large volume chambers and fluorescence microscopy and this permits the definition of lower limits of sperm concentrations for azoospermic samples.

For a copy of the complete article please contact Tara Edwards on 03 98225911 or email [tedwards@gytech.com.au](mailto:tedwards@gytech.com.au).

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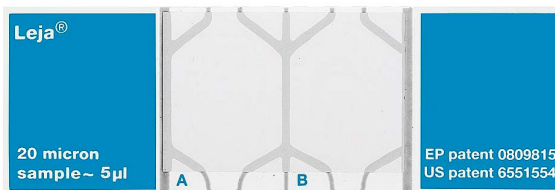
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