



## Boar semen concentration measurement with AndroVision®

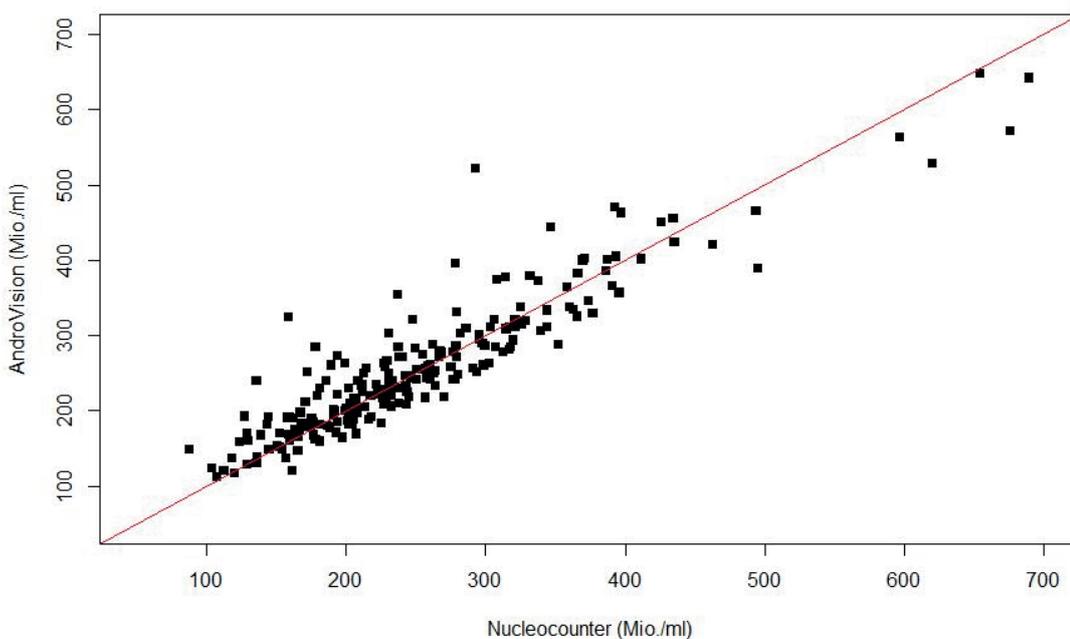
Rudolf Großfeld (Ph.D.), Minitüb GmbH

In order to maximize the use of boars possessing high genetic merit, AI-stations would like to produce as many semen doses as possible from each boar ejaculate. To ensure uniformity of the semen doses, the accurate and precise determination of sperm concentration is a prerequisite for AI-stations, especially as AI-stations are trying to reduce the number of sperm per semen dose<sup>1</sup>.

Validation of measurement systems can be obtained by performing measurements against a reference method. For semen concentration measurement, the NucleoCounter SP-100 (NC) is recommended as such a reference method e.g. by the National Association of Animal Breeders<sup>2</sup> and the Danish National Committee for Pig Production<sup>3</sup>. However, the NC is hardly used in daily production, due to its high running costs, but it nevertheless can serve as a reference for quality control of the sperm number per tube.

Minitube's AndroVision® CASA-System has been thoroughly tested against the NC for semen concentration measurement to ensure a high correctness and precision of the AndroVision® measurements. The aim of these tests is to provide a more economical alternative to the NC without compromising the precision of semen concentration measurement, and offering several additional benefits; one of which is motility measurement during semen concentration determination.

Graph 1 shows the results of such a measurement series. Here, 220 boar ejaculates were evaluated for their raw semen concentration with both the NC and AndroVision®.



Graph 1: Semen concentration measurements of raw boar ejaculates with AndroVision® and NucleoCounter (n=220)

Each dot in this graph represents one measurement. The red line in the graph is the 45-degree line or better, the line of perfect concordance. Measurements/dots very close to or on this line show the same value in AndroVision® and the NC (reference method).

An indicator of the quality of data concordance is the closeness of the measurements to the red line. As can be seen from the graph, nearly all measurements are close to the line which indicates a very good agreement between AndroVision® measurements and NC. A few outliers can be observed. These are most likely due to erroneous pipetting or other preparation flaws which can occur in both methods. These outliers are normal; the fact that the majority of dots in the graph are close to the ideal red line is important.

Visual evaluation of the measurement agreement on a graph is easy, but subjective. Therefore mathematical coefficients should be calculated to describe the measurement agreement numerically.

A coefficient that can be used for this purpose is the Concordance Correlation Coefficient (CCC)<sup>4</sup>. The CCC for the above measurement series is 0.9164. The closer the value approaches 1.0, the better is the agreement between the two measurement methods. In the literature, a coefficient of 0.9164 is described as “almost complete” agreement<sup>5</sup>. An often used and almost identical coefficient to the CCC is the Intra-class Correlation Coefficient (ICC)<sup>6</sup>, which is 0.917 here.

Please note, that the coefficient of determination ( $r^2$ ) is NOT suitable to evaluate an agreement between measurement methods. The  $r^2$  coefficient just describes whether the two measurements have a linear relationship, which one would expect from two methods measuring the same parameter. The  $r^2$  coefficient suffers from the fact that a linear relationship can very well deviate from the line of equality (45-degree line, see above). This can happen for example, if there is a systematic shift in one method due to a mistake in an internal calculation formula, an incorrectly calibrated pipette, or similar. The CCC and ICC consider such deviations as well, in addition to the linearity of the relationship<sup>7</sup> of the two methods.

In summary, the concentration measurement of boar semen with AndroVision® is concordant with an acknowledged reference method. From the statistical point of view, the agreement is almost complete. Boar semen concentration measurement with AndroVision® is therefore a valid and reliable method to determine the number of sperm cells in a boar ejaculate and has a number of additional advantages such as providing more information on a semen sample.

## References

1. Hansen, C. et al. Comparison of FACSCount AF system, Improved Neubauer hemocytometer, Corning 254 photometer, SpermVision, UltiMate and NucleoCounter SP-100 for determination of sperm concentration of boar semen. *Theriogenology* 66, 2188–2194 (2006).
2. Brito, Leonardo F.C. Beckmann, B. et al. NAAB-CSS semen quality control program minimum guidelines. in NAAB Technical Conference on Artificial Insemination and Reproduction 37–41 (2012).
3. ‘Danish Agriculture & Food Council’. Danish Agriculture & Food Council. National committee for pig production guidelines for AI stations: Semen Preservation and Health Control. (2005).
4. Lin, L. I. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45, 255–68 (1989).
5. Koch, R. & Spörl, E. Statistische Verfahren zum Vergleich zweier Messmethoden und zur Kalibrierung: Konkordanz-, Korrelations- und Regressionsanalyse am Beispiel der Augeninnendruckmessung. *Klin. Monbl. Augenheilkd.* 224, 52–57 (2007).
6. Bartko, J. J. The Intraclass Correlation Coefficient as a Measure of Reliability. *Psychol. Rep.* 19, 3–11 (1966).
7. Watson, P. F. & Petrie, A. Method agreement analysis: A review of correct methodology. *Theriogenology* 73, 1167–1179 (2010).

