Dose concentration analysis: a practical study by three laboratories of two boar studs

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Introduction

The concentration of the insemination dose can be influenced by several factors, among them we can mention: calibration of the equipment used, skill of the technicians, the method used for measurement (1,2) (cell counting chamber, spermodensimeter, photometer, CASA, Nucleocounter, etc), the methodologies used by each laboratory that assesses the quality of the dose and the method of correction of the concentration in the dose (volume, motility, % of morphology or a combination of them). These are some sources of variation that alter the concentration of the insemination dose, raising doubts about the accuracy of the techniques/methodologies used in the evaluation of the dose concentration. To verify the sperm concentration in the doses, paired samples could be sent to external laboratories, to evaluate the semen processing system. The aim of diagnosing whether the variations come from the central processing (analysis, dilution, packing) or from the analysis laboratory. Thus, the aim of this study was to compare the sperm concentration of the traditional (80mL) and post-cervical (45mL) doses from Studs 1 and 2 by three laboratories: Laboratory A, Laboratory B and Laboratory C, because for several months the doses presented higher concentration by laboratory A.

Material and methods

The target concentration at traditional insemination (80mL) and post-cervical (45mL) doses should be 2 billion and 1.25 billion viable sperm, respectively. Around 78 males from Central 1 and 104 males from Central 2 were used. The doses, traditional and post-cervical, from the same match were sent weekly (3 weeks) from the centers to three laboratories for analysis of the sperm dose concentration: Laboratory A, Laboratory B and Laboratory C. A total of 235 samples from Stud 1 and 317 samples from Stud 2. The analysis of sperm concentration was performed in a Neubauer chamber in all labs. However, because not all laboratories performed the measurement of the dose volume through weighing or volume, an adjustment was made in the concentration, fixing the values of 45mL and 80mL in the calculation of the sperm concentration, as a way of leveling the sperm concentration for all laboratories. For the concentration of viable sperm, the % of sperm pathologies evaluated in the Magavision® (Magapor, Spain) software was subtracted from the total concentration. Statistical analysis was performed using the R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. The differences were considered significant if $P \leq 0.05$. All descriptive data are expressed as mean ± standard error (SE). “xlsx” library: Adrian Dragulescu and Cole Arendt (2020). xlsx: Read, Write, Format. Excel 2007 and Excel 97/2000/XP/2003 Files. Felipe de Mendiburu and Muhammad Yaseen(2020). Agricolae: Statistical Procedures for Agricultural Research. R package version 1.4.0. https://myaseen208.github.io/agricolae/https://cran.r-project.org/package=agricolae. R package version 0.6.5. https://CRAN.R-project.org/package=xlsx. Analysis of variance (ANOVA) with comparison of means by Tukey's test.

Results and discussion

Our results showed for traditional (80mL) dose and Stud 1, Lab A presented a higher concentration than Lab B and C for total concentration, concentration corrected by volume and concentration corrected by volume and pathology. For post cervical dose (45mL) there was not the

same agreement between the results of the laboratories. Traditional dose in Stud 2 followed the same pattern as in Stud 1, with Lab A presenting high concentration, and for post cervical dose again there was no agreement between labs. We observed the differences between methodologies used in the analysis of sperm concentration by the laboratories, which impacts the results of the doses. Either by assuming the volume printed on the dose package, by mistaken counting of cells in the Neubauer chamber (due to inexperience or overload of hours under the microscope), by pipetting error, in short, there are several factors that influence the analyses. As we are dealing with external laboratories, where there is no management of methodologies (protocols), we adjusted the volume issue, to equalize all laboratories, and for the concentration of viable spermatozoa, the % of pathologies indicated by the Magavision software was subtracted. Analyzes such as sperm concentration and sperm morphology are said to be operator-dependent, as there is a person (employee, trainee) trained to perform it. Therefore, in order to have greater precision in the measurement of sperm concentration, the use of increasingly accurate equipment and the automation of analysis should be sought in an attempt to reduce human errors.

Conclusion

In conclusion, the total agreement of the results between the laboratories is still a controversial topic, as there will be differences between the methodologies used, among the employees who perform the analyses, in the conditions that the samples arrive. And more than that, we need to think that even being the same processing, each dose is a dose. Perhaps the most important is the frequency of sending samples and their behavior over time, which defines the laboratory or not.

References

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Table 1. Mean and standard deviation of the total dose concentration. concentration corrected for dose volume and concentration corrected for dose volume and pathology. of traditional and post-cervical. between laboratories A. B and C. of Stud 1 and 2.

	Stud 1		**Stud 2**	
Laboratory	**Total Dose Concentration**			
	Traditional (80mL)	**Post-cervical (45mL)**	**Traditional (80mL)**	**Post-cervical (45mL)**
A	2.82 ± 0.34 a	1.66 ± 0.19 a	3.02 ± 0.32 a	1.65 ± 0.15 a
B	2.36 ± 0.46 b	1.47 ± 0.30 b	2.69 ± 0.81 b	1.54 ± 0.26 b
C	2.37 ± 0.63 b	1.71 ± 0.58 a	2.68 ± 0.44 b	1.60 ± 0.45 a
Laboratory	**Concentration corrected for dose volume**			
A	2.92 ± 0.34 a	1.74 ± 0.2 a	3.04 ± 0.34 a	1.78 ± 0.18 a
B	2.46 ± 0.5 b	1.54 ± 0.32 b	2.77 ± 0.46 b	1.61 ± 0.28 b
C	2.37 ± 0.63 b	1.71 ± 0.58 ab	2.68 ± 0.81 b	1.60 ± 0.45 b
Laboratory	**Concentration corrected for dose volume and pathology**			
A	2.48 ± 0.33 a	1.46 ± 0.20 a	2.46 ± 0.032 a	1.39 ± 0.16 a
B	2.10 ± 0.49 b	1.29 ± 0.27 b	2.22 ± 0.36 b	1.29 ± 0.21 a
C	2.04 ± 0.60 b	1.40 ± 0.48 ab	2.15 ± 0.67 b	1.30 ± 0.36 a

Different letters on column mean statistical difference (P<0.01) between labs.