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COMPARATIVE ASSESSMENT OF BULL SPERM MOTILITY AND CONCENTRATION: CONVENTIONAL METHODS VERSUS PORTABLE ANDROSCOPE CASA SYSTEM

Perbandingan Pengujian Motilitas dan Konsentrasi Sperma Sapi: Metode Konvensional versus Sistem Androscope CASA Portabel

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ABSTRACT
Operational implementation, such as laboratory staff and equipment, differ for each frozen semen producer, resulting in variability in the quality assessment of the frozen semen produced. Conventional assessments subject to subjectivity, human error, and high variability. Computer-assisted semen analysis (CASA) is considered more objective. This study aimed was to conduct a comparative analysis of motility and concentration assessment of frozen semen sperm using conventional and the portable CASA AndroScope. Twenty-one laboratory assistants from 21 national and regional frozen semen producers participated in this study. Sperm motility and concentration were assessed conventionally (using a Neubauer chamber) and AndroScope was used with five replicates. The results of the comparison of sperm motility scores revealed significant differences ($p<0.05$) between conventional examination and using AndroScope. The results of the comparison of the sperm concentration calculations revealed no significant difference ($p>0.05$) between the calculations performed using the Neubauer chamber and AndroScope. Assessment with the AndroScope is considered reliable and can replace conventional assessment in assessing motility and sperm concentration, thereby improving and standardizing the assessment performed by frozen semen producers.

Keywords: *AndroScope, CASA, Semen analysis, Sperm Concentrations, Sperm Motility*

ABSTRAK
Implementasi operasional seperti sumber daya manusia laboratorium dan peralatan pada masing-masing produsen semen beku berbeda sehingga menghasilkan variabilitas pada penilaian mutu semen beku yang diproduksi. Pengujian secara konvensional memiliki subjektifitas dan human error serta variabilitas yang tinggi. Penilaian menggunakan computer assisted sperm analysis (CASA) dinilai lebih objektif. Tujuan dari penelitian ini ialah melakukan komparatif analisis penilaian motilitas dan konsentrasi sperma semen beku secara konvensional dan menggunakan CASA portable AndroScope. Laboran yang berpartisipasi berjumlah 21 orang berasal dari 21 produsen semen beku nasional dan daerah. Pengujian motilitas dan konsentrasi sperma dilakukan secara konvensional (manual dan menggunakan neubauer chamber) dan menggunakan AndroScope dengan lima kali ulangan. Hasil perbandingan penilaian motilitas sperma yang diperoleh menunjukkan perbedaan yang signifikan ($p<0,05$) antara pemeriksaan secara conventional dan menggunakan AndroScope. Hasil perbandingan perhitungan konsentrasi sperm menunjukkan tidak ada perbedaan signifikan ($p>0,05$) antara perhitungan menggunakan neubauer chamber dan AndroScope. Pengujian menggunakan AndroScope

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dinilai reliabel dan dapat menggantikan pengujian secara konvensional dalam penilaian motilitas dan konsentrasi sperma, sehingga meningkatkan dan menstandardisasi pengujian yang dilakukan oleh produsen semen beku.

Kata Kunci: *AndroScope, Analisis Semen, CASA, Konsentrasi Sperma, Motilitas Sperma*

INTRODUCTION

In Indonesia, the production of frozen semen for artificial insemination (AI) is carried out by the National Artificial Insemination Center (NARC) and Regional Artificial Insemination Centers (RAIC). This task is performed by personnel working in the laboratories of frozen semen producers. Their primary duty is to generate frozen semen from high-quality males (Prabuwisudawan et al. 2018). The production and distribution processes adhere to the Regulation of the Minister of Agriculture (MOA) of the Republic of Indonesia No. 10/2016.

MOA No. 10/2016 acts as a directive for every producer of frozen semen regarding the creation and distribution of their products. This MOA is integrated into the standard operating procedures (SOPs) of each producer. While frozen semen producers typically adhere to similar SOPs, variations exist in their production goals, operational methods, and the way they apply these procedures at their respective facilities. According to MOA No. 10/2016, frozen semen production units that have not adopted ISO 17025:2008 in their laboratories must perform interlaboratory comparisons with laboratories that have implemented this standard.

Assessing the quality of both fresh and frozen semen is crucial for evaluating frozen semen quality. However, the way this process is carried out, including the involvement of human resources, technicians, and equipment at each facility, varies, leading to differences in the quality assessment of frozen semen production, as documented by Gacem et al. (2020) and Singh et al. (2021). Traditionally, sperm motility is assessed using conventionally observation with a binocular microscope or computer-assisted sperm analysis (CASA). The concentration of fresh semen is measured using a spectrophotometer, whereas the frozen semen concentration is determined using a counting chamber, such as a Neubauer chamber or CASA. It is essential that individuals conducting semen assessments be properly trained and standardized, as conventional assessment methods are inherently subjective, prone to human error, and exhibit significant variability (Cardeal et al. 2017).

The precision of semen quality evaluation is adversely affected by conventional evaluation methods. In contrast, computer-assisted sperm analysis (CASA) is regarded as more objective (Daloglu and Ozcan 2017; Luther et al. 2020; Finelli et al. 2021). CASA devices are available from various manufacturers, including Minitube (Germany), and in different formats, such as laboratory-based and portable models. However, the substantial financial investment required for the equipment and operation of laboratory-based CASA is the main barrier preventing some frozen semen producers from accessing it, especially in resource-limited agricultural and industrial contexts (RAICs).

Portable CASA devices, such as AndroScope, offer a practical alternative because of their affordability, user-friendliness, compact size, and portability, allowing for semen quality assessment at various locations and times. This study aimed to compare the motility assessment and sperm concentration of frozen semen using conventional methods and AndroScope.

MATERIALS AND METHODS,

Location and Time

The study was conducted from September 2023 to November 2024 at the Central Java Regional Artificial Insemination Center (RAIC) in Ungaran, Central Java, and at the School of Veterinary Medicine and Biomedicine, IPB University, Indonesia.

Materials

Frozen semen from the Ungaran RAIC was used in the present study. Other materials used included glass slide, cover

glasses, microtubee, formal saline, and physiological saline. The tools used in the study included a light microscope, micropipette, straw scissors, CASA portable (AndroScope, Minitube, Germany), Neubauer chamber, water bath, and heating table (37°C).

Methods

The frozen semen was thawed in a water bath (37℃) for 30 s. The straw was cut at both stoppers, and the semen was placed in a microtube and kept in a water bath for observation.

Conventional motility test

A total of 4-10 µL of thawed semen was place on a warmed microscope slide and covered with a coverslip. Examination under a microscope equipped with a warming table (37℃) was performed starting from magnifications of 10×10, 20×10, and 40×10. The evaluation was carried out in a minimum of 5 -10 fields of view, with sperm observed in a field of view containing only 10-20 cells per field. The assessment was performed by examining the proportion or ratio of sperm that moved forward (progressive) compared to other motions. Values are expressed as percentages (%).

Conventional Sperm Concentration Test

Formol saline (990 µL) was added to the microtube. The frozen semen was thawed, and 10 µL was taken up with a micropipette. The outside of the tip was cleaned with a tissue. The semen was placed in a diluent microtube and rinsed several times to ensure that all the semen in the microtip was in the diluent solution. The solution was homogenized by rotating the microtube in a figure-eight motion. The solution was adjusted to a final volume of 8-10 µL and placed in the chamber (Handayani et al. 2021).

Sperm number per mL = N x 5 x FP x10,000

Description:

N : average number of sperm in 2 chambers

FP : dilution factor

‘5’ : calculation correction factor (5 out of 25 boxes)

‘10,000’ : correction factor 0.0001 ml per chamber

Motility and concentration testing via AndroScope

Motility and sperm concentration calculations via CASA portable - AndroScope were performed simultaneously with Leja® slides. The AndroScope was attached to a laptop or tablet, the software was run, and live image flashes were set until the optimal analysis temperature was attained. A total of 3 µL frozen and thawed semen was collected and placed on a special Leja® object slide. The data were recorded and tabulated accordingly.

Data Analysis

The results are presented as mean ± standard deviation. Student's t-test was used to analyze the significance of differences in sperm motility and concentration between the two groups and evaluation methods. Differences were considered statistically significant at $P \leq 0.05$. Analyses were performed using IBM SPSS® Statistics version 27.0 (IBM Corp., Armonk, NY, US).

RESULTS AND DISCUSSION

The comparative results of the sperm motility assessment revealed significant differences ($p<0.05$) between the conventional examination and CASA using AndroScope (Table 1). These findings demonstrate the influence of the sperm motility assessment method on the results obtained. Conventional assessments are prone to operator subjectivity, human error, and intra-operator variability. This is believed to affect the accuracy of the assessment results, which ultimately affects the decision-making for quality testing of the produced frozen semen. It is common for sperm to be over- or under-scored during conventional grading. According to Finelli et al. (2021), this is because the human eye is attracted to the movement of the sperm and the human eye is unable to distinguish between different types of sperm motility, whereas the camera and tracking system in CASA can more clearly detect and classify the type of movement.

Table 1. Comparation of sperm motility evaluated by the staff laboratory with those evaluated via conventional assessment and AndroScope.

Staff Laboratory	Sperm Motility (%) Evaluation technique*		p-value
	Conventional	AndroScope	
1	47.50	53.58	0.028
2	43.75	66.27	0.009
3	40.00	57.49	0.014
4	46.00	62.55	0.016
5	49.00	71.98	0.008
6	36.00	67.46	0.003
7	38.00	60.59	0.009
8	42.00	59.36	0.014
9	40.00	66.29	0.005
10	51.00	68.92	0.014
11	36.00	57.31	0.001
12	43.00	61.50	0.012
13	37.00	61.29	0.007
14	25.38	53.70	0.004
15	28.00	47.19	0.011
16	27.00	52.11	0.006
17	20.00	44.74	0.007
18	30.00	47.96	0.014
19	38.13	69.44	0.003
20	23.33	57.90	0.001
21	33.33	65.88	0.002
(n = 105)	**36.87 ± 1.89[a]**	**59.69 ± 1.68[b]**	**<0.0093**

*Each contributing five repetitions.

The analysis of the sperm concentration revealed no significant difference (P< 0.05) between the results obtained using the Neubauer chamber and the CASA portable AndroScope (Table 2). These findings suggests that CASA portable AndroScope calculations align with the gold standard method, which serves as a benchmark for determining the sperm concentration. Ismawatie et al. (2021) also reported similar findings, with no significant difference (P< 0.05) in conventional sperm concentration calculations using CASA. The CASA system is a reliable alternative for assessing semen quality in bulls.

Table 2. Comparison of sperm concentrations evaluated by the staff laboratory with those evaluated via conventional assessment and AndroScope.

Staff Laboratory	Sperm Concentration (×10^6 ml) Evaluation Technique*		p-value
	Conventional	AndroScope	
1	27.23	21.52	0.39
2	32.83	20.80	0.04
3	29.44	37.35	0.38
4	31.63	26.48	0.38
5	24.56	20.88	0.79
6	31.48	28.50	0.85
7	29.06	27.56	0.74
8	32.09	31.43	0.87
9	34.38	33.86	0.91

Staff Laboratory	Sperm Concentration (×10^6 ml) Evaluation Technique*		p-value
	Conventional	AndroScope	
10	35.41	28.08	0.46
11	30.38	34.07	0.79
12	40.62	24.83	0.02
13	37.25	33.99	0.75
14	27.03	34.12	0.38
15	19.14	15.19	0.81
16	27.03	28.78	0.78
17	35.73	20.15	0.02
18	31.09	16.16	0.03
19	61.88	71.44	0.05
20	14.69	33.61	0.01
21	28.44	16.41	0.03
(n = 105)	**31.49 ± 1.97[a]**	**28.81 ± 2.58[a]**	**0.45**

*Each contributing five repetitions.

Utilizing CASA enhances analytical efficiency and can increase the dependability of the outcomes (Finelli et al. 2021). According to Klimowicz et al. (2008), employing CASA for evaluation enables an objective, concurrent, swift, and precise analysis of various semen quality metrics, including sperm concentration, total motility, percentage of progressive motility, velocity, movement linearity, and morphology. The objective evaluation facilitated by CASA also permits the computation of quantitative parameters that are unattainable through conventional subjective assessments (Daloglu and Ozcan 2017; Luther et al. 2020). CASA provides faster and more precise results for main variable such as sperm motility and concentration in routine sperm analysis (Schubert et al. 2019).

The conventional assessment of semen is considered standardized when performed by a competent and trained operator in an accredited laboratory and participating in a proficiency program monitored by an external body. As with conventional assessments, errors in assessment via CASA can be caused by operators. Finelli et al. (2021) and Ratnawati et al. (2019) described several factors that affect assessment via CASA, including CASA settings, semen diluent, sperm concentration, sample preparation, test performance and operator. Assessment via CASA should be performed consistently by an experienced operator (laboratory assistant) to minimize the error factors or variations in the analysis results. This is related to the operator's expertise in sample preparation and selection of the field of view on the screen, as well as the timing of the evaluation (Dincer et al. 2024).

Adjusting CASA parameters, including frame selection, ideal temperature settings, and chamber type, is crucial for conducting accurate and consistent analyses, thereby reducing error factors during evaluations (Gacem et al. 2020). Furthermore, variations in the type and quantity of diluent components can influence the sperm motility. The use of unsuitable diluents can lead to the formation of other particles, such as spherules or debris, which may be smaller, the same size, or even larger than the sperm. This can skew CASA assessments, as these particles might be mistaken for static sperm because of their similar sizes. The result is a motility value that is biased or does not accurately represent the true motility. The presence of these particles also restricts sperm movement, leading to reduced motility (Ratnawati et al. 2019).

Sample preparation is related to the concentration or density of sperm during evaluation. According to Ratnawati et al. (2019), optimal results in sample preparation, which begins with pipetting (the method of extracting samples), are achieved if mixing and sampling (the method of obtaining samples) performed a few minutes after collection with a medium density level. This approach prevented an overly dense concentration of sperm in the sample. When the sperm concentration was high, the heads

tended to collide and stick together along their path. A dense population limits sperm movement, which can diminish the true motility potential. Lower concentrations provide more accurate results in motility assessments via CASA, as the path of each sperm can be precisely captured and analyzed.

CONCLUSION

The results indicated that laboratory assistant of AIC conducted a comparative analysis, revealing notable differences in motility assessment between conventional methods and the CASA portable AndroScope. The sperm concentration determined by portable AndroScope CASA was consistent with the gold standard calculation used as a reference for sperm concentration measurement. The CASA portable AndroScope test is regarded as more objective than the other methods.

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