A Comparison study between manual semen analysis with that of computer assisted semen analysis.

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ABSTRACT :

Objectives: The study aimed to check the quality of the computer-assisted semen analysis (CASA) system compared to the reference manual method and standardisation of the computer-assisted semen assessment. **Material and methods**: The study was conducted between October 2023 and March 2024 at MMCRI, Mysore. The samples underwent manual and computer-assisted concentration, motility, and morphology assessment. A total of 68 samples were examined twice: manually, according to the 2021 WHO recommendations, and with CASA, using the program settings provided by the manufacturer. The p-value of $p < 0.05$ was considered statistically significant. **Results:** Statistically significant differences were found among all investigated sperm parameters, except for non-progressive motility, as measured with CASA and manually. In the group of patients where all analyses with each method were performed twice on the same sample, we found no significant differences between both assessments of the same probe, neither in the samples analysed manually nor with CASA. However, the standard deviation was higher in the CASA group. **Conclusions**: Our results suggest that computer-assisted semen analysis requires further improvement for a broader application in clinical practice.

Keywords: computer-assisted semen analysis, CASA, Motility, Morphology assessment.

INTRODUCTION:

Andrological problems are faced by 48 million couples and 186 million individuals globally.[1] Infertility affects around one in eight couples of reproductive age, with a male factor being solely responsible in 20% and contributory in an additional 30% of cases. [2] A malefactor could be a primary or contributing cause in approximately 50% of couples.[3]

The main issues include male infertility, male contraception, hypogonadism, erectile dysfunction, and male ageing. There are also other emerging concerns, such as testicular cancer, BPH, prostate cancer, delayed puberty, family planning, cryopreservation of semen and testicular tissues, hormone replacement therapy, and forensic paternity issues. A wide range of treatment options are now available for procedures such as varicocele, vas deferens, and epididymis reconstruction using microsurgical techniques. These treatments also include assisted intrauterine insemination, in vitro fertilisation, intracytoplasmic sperm injection (ICSI), as well as a combination of antibiotics and anti-inflammatory agents to improve semen quality in chronic tobacco smokers. The semen characteristics provide relevant information about male infertility. Having a standardised approach for semen analysis is, therefore, crucial to limit the variation in obtaining and processing ejaculates. However, manual semen analysis can be time-consuming and tedious.[4]

Given the need, the development of computer-aided semen analysis started in the 1980s.[5] A computer-based solution is beneficial for automating and standardising semen analysis, and to date, many modifications and sophisticated models have emerged. The precision and accuracy of sperm concentration estimates are determined primarily by technician skills and limitations inherent to the method used, including equipment specifications and setup in the case of automated methods.[6] So, in our study, we tended to manually analyse and compare the results of semen analysis with those of CASA.

OBJECTIVES OF THE STUDY:-

The study assesses and compares the manual semen analysis with computer-aided semen analysis.

MATERIALS AND METHODS:

Data for the present study is obtained from the person with primary or secondary infertility coming to the Department of Pathology, Mysore Medical College and Research Institute from October 2023 to March 2024 (6 months). The clinical details were collected from the subject. All men with primary and secondary infertility, irrespective of age, in the department of pathology, MMCRI. All azoospermia and semen volumes less than 1 ml are excluded from the study.

The participants were to collect the semen sample by masturbation after 3-5 days of sexual abstinence. Specimens will be collected into a sterile plastic container and examined after 30 min liquefaction. Physical examinations such as volume, colour, viscosity, pH and microscopic examination are to be done for various qualities and quantities.

Manual and computer method interpretation:

- **Motility:**

We will assess the motility parameters on the freshly wet mount slide, such as rapid progressive motility, slow progressive motility, nonprogressive, and immotile percentage, which can be subjective most of the time.

- **Viability:**

We will assess the viability of these sperms with one drop of each eosin ink and semen. The non-viable sperms will be stained pink as the ink penetrates within the acrosome after its structural damage.

- **Counting:**

The sperm count can be done using the Neubauer chamber. Dilution is done in a ratio of 1:20, and the corner four chambers are assessed for the sperm under 40X magnification.

The total sperm concentration will be multiplied by the total volume of semen collected.

- **Morphology:**

The semen smear is stained with Giemsa stain and assessed for its various morphology under 40x.

STATISTICAL METHODS

Data will be analysed using the SPSS 22.0 version, and appropriate parametric and non-parametric tests, e.g., the t -t-students test, were used to compare the results obtained manually and CASA. Will be employed according to the study variable. P value < 0.05 was considered to be statistically significant.

RESULTS:

68 semen samples were assessed between the ages of 24 and 46, with a mean age of 32.9. There were 49 cases of primary and 19 cases of secondary infertility. The primary infertility versus secondary infertility ratio is 2:6 (figure1). Based on the final report, most cases were of normozoospermia, and the least were oligoasthenozoospermia and azoospermia (Figure 2). The median liquefaction time was 20 minutes. As soon as the semen was liquified, various parameters were examined first manually and then with the aid of CASA (Table 1). Statistically significant differences between both methods for multiple parameters were reviewed except for the non-progressive motility and morphology.

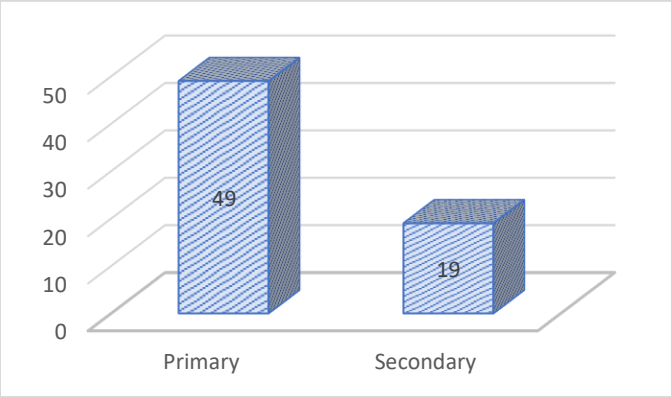

Figure 1: Distribution of cases based on type of infertility

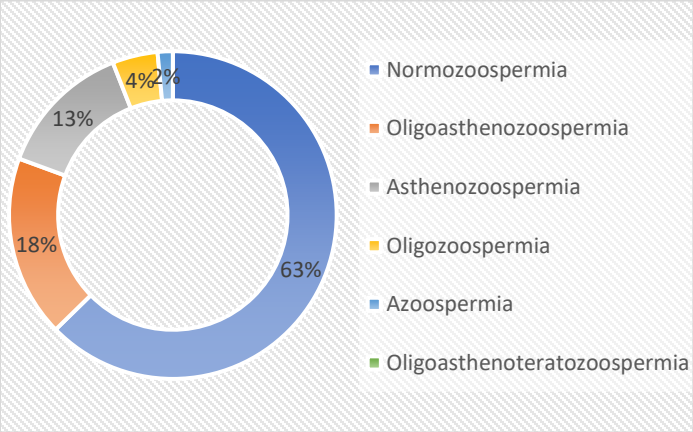

Figure 2: Distribution of cases based on the final report

Table 1: Comparison of sperm parameters obtained by manual and CASA analysis of 68 cases.

	Concentration		**Progressive motility (%)**		**Non-progressive motility (%)**		**Immotile (%)**		**Morphology (%)**	
	M	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
mean	28	31	27	29	34	31	38	35	4	
SD	20	24	14	16	15	10	18	23	1	3
p	<0.05%		<0.05%		>0.05%		< 0.05%		>0.05%	

CASA- computer-assisted sperm analysis, M – manual assessment, C- computer assessment, SD-standard deviation

DISCUSSION:

According to the WHO and ESHRE manual, semen analysis is the gold standard method to determine male infertility. The WHO 2021 semen reference parameters were taken into account. Our results and previous studies show that computer-assisted sperm analysis needs further studies.

We noticed a higher standard deviation in the sample processing in CASA. The technicians using CASA lacked specialised training and should gain experience in semenology and its analysis. Utilising Leja chambers and the producer setting in CASA was crucial in reproducing the final report. Our study highlights the discrepancies even after implementing these measures. Almost in all cases, concentrations were higher in the CASA compared to the manual studies. The motility comparison reveals a significant difference except for the non-progressive motility. All CASA values are higher when observed in CASA, similar to Cooper et al.'s study.[7]

The progressive motility values were higher in CASA. This may be due to a technical glitch where the computer cannot differentiate the velocity of the gametes when they cross. It is not able to assess the flagellar beating properly.[8]

Automated assessment takes longer than manual evaluation of morphology. The standard deviation values of CASA were significantly higher than the manual method's. These limitations of CASA have been acknowledged, and many producers are currently focused on improving the system by teaching better Brownian movement filtering, illumination control, etc.

CONCLUSION:

CASA systems offer several advantages in speed, data storage, and minimal technician training. However, discrepancies between the results and manual assessment indicate that further development is needed for CASA to be used exclusively for infertility testing.

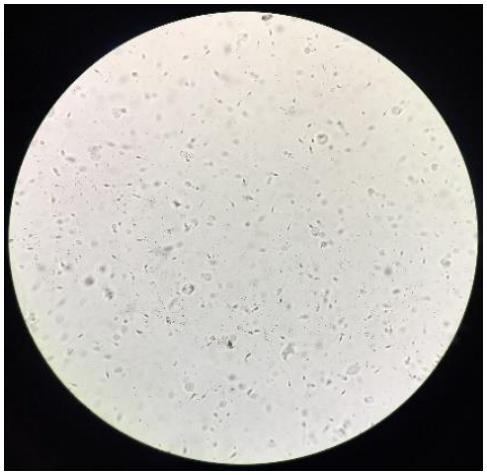

Figure 1: Wet mount at 40x

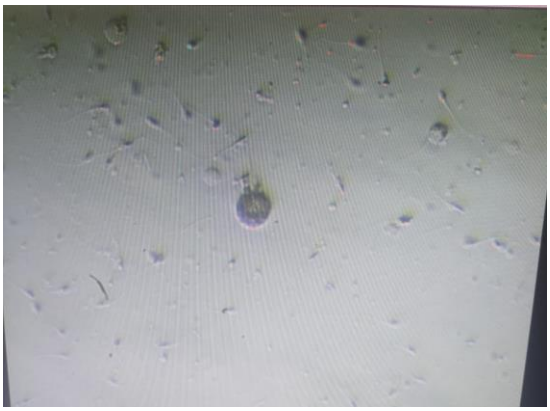

Figure 2: Motility study on CASA

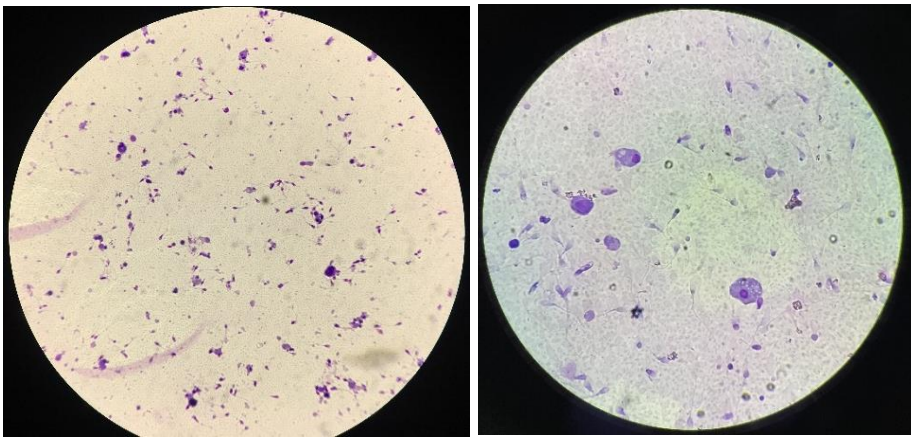

Figure 3: Giemsa stain of sperms at 40x and 100x

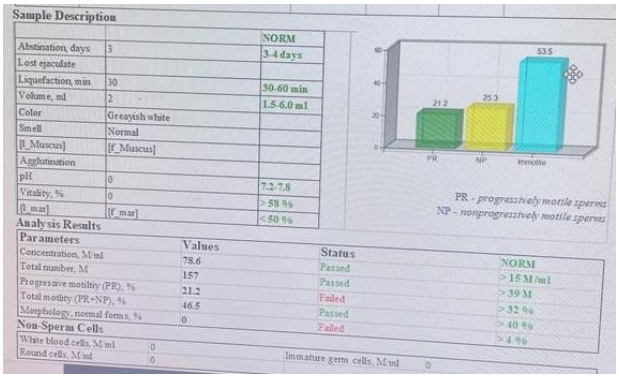

Figure 6: Report display in CASA

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