Reproduction & Fertility

Reproduction & Fertility (2025) **6** e250014
https://doi.org/10.1530/RAF-25-0014

Received 7 February 2025
Accepted 22 April 2025
Available online 22 April 2025
Version of Record published 2 May 2025

RESEARCH

Artificial intelligence model for the assessment of unstained live sperm morphology

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Abstract

Traditional sperm morphology assessment requires staining and high magnification (100×), rendering sperm unsuitable for further use. We aimed to determine whether an in-house artificial intelligence (AI) model could reliably assess normal sperm morphology in living sperm and compare its performance with that of computer-aided semen analysis and conventional semen analysis methods. In this experimental study, we enrolled 30 healthy male volunteers aged 18–40 years at the Songklanagarind Assisted Reproductive Centre, Songklanagarind Hospital. We developed a novel dataset of sperm morphological images captured with confocal laser scanning microscopy at low magnification and high resolution to train and validate an AI model. Semen samples were divided into three aliquots and assessed for unstained live sperm morphology using the AI model, whereas computer-aided and conventional semen analysis methods evaluated fixed sperm morphology. The performance of our in-house AI model for evaluating unstained live sperm morphology was compared with that of the other two methods. The in-house AI model showed the strongest correlation with computer-aided semen analysis (r = 0.88), followed by conventional semen analysis (r = 0.76). The correlation between computer-aided semen analysis and conventional semen analysis was weaker (r = 0.57). Both the in-house AI and conventional semen analysis methods detected normal sperm morphology at significantly higher rates than computer-aided semen analysis. The in-house AI model could enhance assisted reproductive technology outcomes by improving the selection of high-quality sperm with normal morphology. This could lead to better outcomes of intracytoplasmic sperm injections and other fertility treatments.

Lay summary

We evaluated a new in-house AI model for assessing the shape and size (morphology) of live sperm without staining and performed comparisons with computer-aided semen analysis and conventional semen analysis, which require sperm to be fixed and stained before analysis. This new method of assessing unstained, live sperm is significant because it facilitates viable sperm selection for use in assisted reproductive technology immediately after assessment, ultimately contributing to improved fertility outcomes. The AI model allowed sperm morphology assessments with significantly improved accuracy and reliability. By using high-resolution images and advanced microscopy, the AI model could detect subcellular features. This AI model could be an effective tool in clinical settings, because it minimizes subjectivity and improves sperm selection for assisted reproductive technologies, potentially leading to higher success rates in infertility treatments. Further research can refine the model and validate its effectiveness in diverse clinical environments.

Keywords: assisted reproductive technology; artificial intelligence; confocal microscopy; semen analysis; sperm morphology

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Introduction

Embryo quality in assisted reproductive technology (ART) is influenced by both oocyte and sperm quality. While advanced maternal age, particularly ≥35 years, significantly affects oocyte quality (Braude *et al.* 1988), male factors contribute to approximately 50% of infertility cases (Cherouveim *et al.* 2023). Normal sperm morphology is associated with intact DNA and favourable clinical outcomes, whereas high sperm DNA fragmentation adversely affects fertilisation and embryonic development (Ribas-Maynou *et al.* 2021). However, conventional sperm morphology assessment requires staining and high magnification (100×), rendering sperm unsuitable for further procedures. Advanced techniques, such as morphological examination of multiple sperm organelles for intracytoplasmic morphologically selected sperm injection (IMSI), also necessitate magnifications of >600× and are time-intensive procedures (Itoi *et al.* 2022).

However, conventional sperm assessment methods often involve subjectivity, which can impact result interpretation and treatment planning. Artificial intelligence (AI) offers objective, automated and accurate sperm analysis, including the assessment of motility and morphology. It minimizes the subjectivity observed with conventional semen evaluation methods and may enhance sperm selection in the ART process (Nashed *et al.* 2025). Javadi & Mirroshandel (2019) reported that AI systems can assess sperm morphology at low magnification without staining. These models require extensive training and validation datasets. Available datasets include the Human Sperm Morphology Analysis (HSMA-DS) dataset, with 1,475 images at 40–60× magnification and its subset, the modified HSMA dataset, containing 1,540 images of sperm heads (Javadi & Mirroshandel 2019). The SVIA dataset offers 101 videos and 4,041 low-resolution images of unstained sperm (Chen *et al.* 2022). In contrast, datasets such as the Human Sperm Head Morphology, SCIAN-MorphoSpermGS and Sperm Morphology Image Data Set focus on stained sperm (Riordon *et al.* 2019, Ilhan *et al.* 2020*a*,*b*, Iqbal *et al.* 2020). Nevertheless, limitations in existing datasets – such as low resolution, limited sample size and insufficient categories – impede effective machine learning model development.

We introduced a high-resolution, low-magnification dataset of sperm morphology images obtained using confocal laser scanning microscopy. Then, we trained an AI model to evaluate unstained live sperm morphology and compared its performance with that of computer-aided semen analysis (CASA) and conventional semen analysis (CSA) used to assess the morphology of stained and fixed sperm.

Materials and methods

Study participants

Thirty healthy volunteers aged 18–40 years were enrolled between February 1, 2024 and February 29, 2024, at the Songklanagarind Assisted Reproductive Center, Songklanagarind Hospital. Personal information was recorded, and participants were informed to maintain 2–7 days of sexual abstinence before providing an ejaculate for analysis, with reporting of any wasted fractions. The sample size was calculated using the three-dependent means method based on pilot study results (n = 3), with a mean of 3.3 for the AI method, a standard deviation (SD) of 2.9 and a mean of 1.3 for the CASA method. Exclusion criteria included abstinence periods outside 2–7 days, sperm collected via intercourse or in improper containers, use of spermicidal agents, high sperm viscosity and semen volume <1.4 mL.

Sample collection

Thirty semen samples were collected through masturbation in sterile containers in the laboratories. Liquefaction was checked within 30 min of ejaculation, and conventional sperm assessment was performed according to the Björndahl guidelines (Bjorndahl *et al.* 2016). Specimens were preserved at 37°C before and during sperm motility assessment. Each sample was aliquoted into three tubes.

Semen analysis

Semen volume was measured by weighing. Viscosity was measured via a wide-bore pipette, and pH was measured using a pH test strip with a well-mixed semen drop.

Sperm parameters, specifically concentration and motility, were analysed using the CASA system (IVOS II; Hamilton Thorne, USA). Each assessment involved two replicates to confirm consistency.

Wet preparations were made with a 6 μL semen drop on LEJA slides (026855, SC-20-01-C) under a 4 × 25 mm coverslip, which created a 20 μm preparation depth. At least 200 spermatozoa were evaluated across five microscopic fields per replicate. Sperm morphology was analysed using in-house AI, CASA and CSA methods, adhering to the WHO Laboratory Manual for the Examination and Processing of Human Semen (sixth edition) (World Health Organization 2021).

Assessment of sperm morphology by the in-house AI model

Dataset

The sample was dispensed as a 6 μL droplet onto a standard two-chamber slide with a depth of 20 μm

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(Leja®). To create the datasets, sperm images were captured using a confocal laser scanning microscope (LSM 800) at 40× magnification in the confocal mode (LSM, Z-stack). The Z-stack interval was 0.5 μm, covering a total range of 2 μm. Five slides were generated, each with a frame time of 633.03 ms and a size of 512 × 512 pixels. The image size per slide was 159.7 × 159.7 μm. At least 200 sperm images (each containing 2–3 sperm per capture) were collected per sample.

Embryologists and researchers manually annotated well-focused sperm images with bounding boxes for each sperm using the LabelImg program. The coefficient of correlation between embryologists and researchers for the detection of normal sperm morphology was 0.95, while that for the detection of abnormal sperm morphology was 1.0. There is no well-established standard for unstained sperm morphology assessment under low magnification. To evaluate sperm morphology, we used algorithms regarding the WHO Laboratory Manual for the Examination and Processing of Human Semen (sixth edition) (World Health Organization 2021). Each sperm image was categorised into nine datasets, which included normal sperm with a smooth oval head, a length-to-width ratio of 1.5–2, no vacuoles, a slender and regular neck, a uniform calibre along the length of the tail and cytoplasmic droplets less than one-third of the sperm head. The abnormal dataset included an abnormal sperm head characterised by a tapered, amorphous, pyriform or round shape; an observable vacuole; an aberrant sperm neck; or an abnormal sperm tail. Normal morphology was confirmed when the sperm met the criteria for normal morphology in all five frames (Fig. 1A and B).

In-house AI model and machine learning method

We used a machine learning approach to assess sperm morphology by developing a deep learning model for sperm classification. A flowchart illustrating the development of the AI model for assessing sperm morphology is shown in Fig. 2. For this experiment, we selected the ResNet50 transfer learning model, a deep neural network designed for image classification tasks. We trained this model on our dataset to minimise the difference between the predicted and actual labels. The model performance was evaluated using a separate test dataset that was not used during training. This evaluation assessed the effectiveness of the deep learning model in classification of the sperm morphology.

Our dataset contained 21,600 images, with 12,683 annotated as unstained sperm. The machine learning model was tested on 900 batches of previously unseen images, achieving a test accuracy of 0.93 after 150 epochs. The training model used a subset of 9,000 images derived from 32 pattern samples in each group: 4,500 for normal sperm morphology and 4,500 for abnormal sperm morphology. The model exhibited a precision of 0.95 and recall of 0.91 for detecting abnormal sperm morphology and a precision of 0.91 and recall of 0.95 for normal sperm morphology. The model's processing time was approximately 139.7 s for 25,000 images, resulting in an average prediction time of approximately 0.0056 s per image.

Assessment of sperm morphology using CASA

The sample was allowed to air-dry on a glass slide and stained with Diff-Quik stain (Romanowsky stain variant), and at least 200 sperm were assessed under 100× magnification using the CASA system (IVOS II, Hamilton Thorne). Normal sperm morphology was evaluated according to the Tygerberg strict criteria implemented in the DIMENSIONS II Sperm Morphology Analysis software (Hamilton Thorne Inc 2019) based on the default settings.

Assessment of sperm morphology using CSA

The specimen was smeared onto a glass slide and air-dried to facilitate fixation before staining with Diff-Quik stain. At least 200 sperm were evaluated under 100× magnification using a compound light microscope (ECLIPSE E200; Nikon, Japan). The laboratory participated in external quality assessment (EQA) schemes administered by the Association of Thai Embryologists, ensuring the standardisation and validation of the semen analysis protocols utilised in the study in accordance with the established andrology and embryology guidelines. This included EQA for sperm concentration, motility and morphology.

Statistical analysis

Data were analysed using the STATA version 12 (StataCorp, USA). Baseline characteristics and semen parameters are presented as the mean ± SD for normally distributed data and the median ± interquartile range (IQR) for non-normally distributed data. Poisson regression was used to assess the differences between the in-house AI model and the other semen analysis methods (CASA and CSA). Pairwise correlations were used to evaluate the correlation between the percentage of normal sperm morphology detected by the in-house AI model and that detected by CASA and CSA. *P*-values <0.5 were considered statistically significant.

Ethics approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Declaration of Helsinki of 1964 and its later

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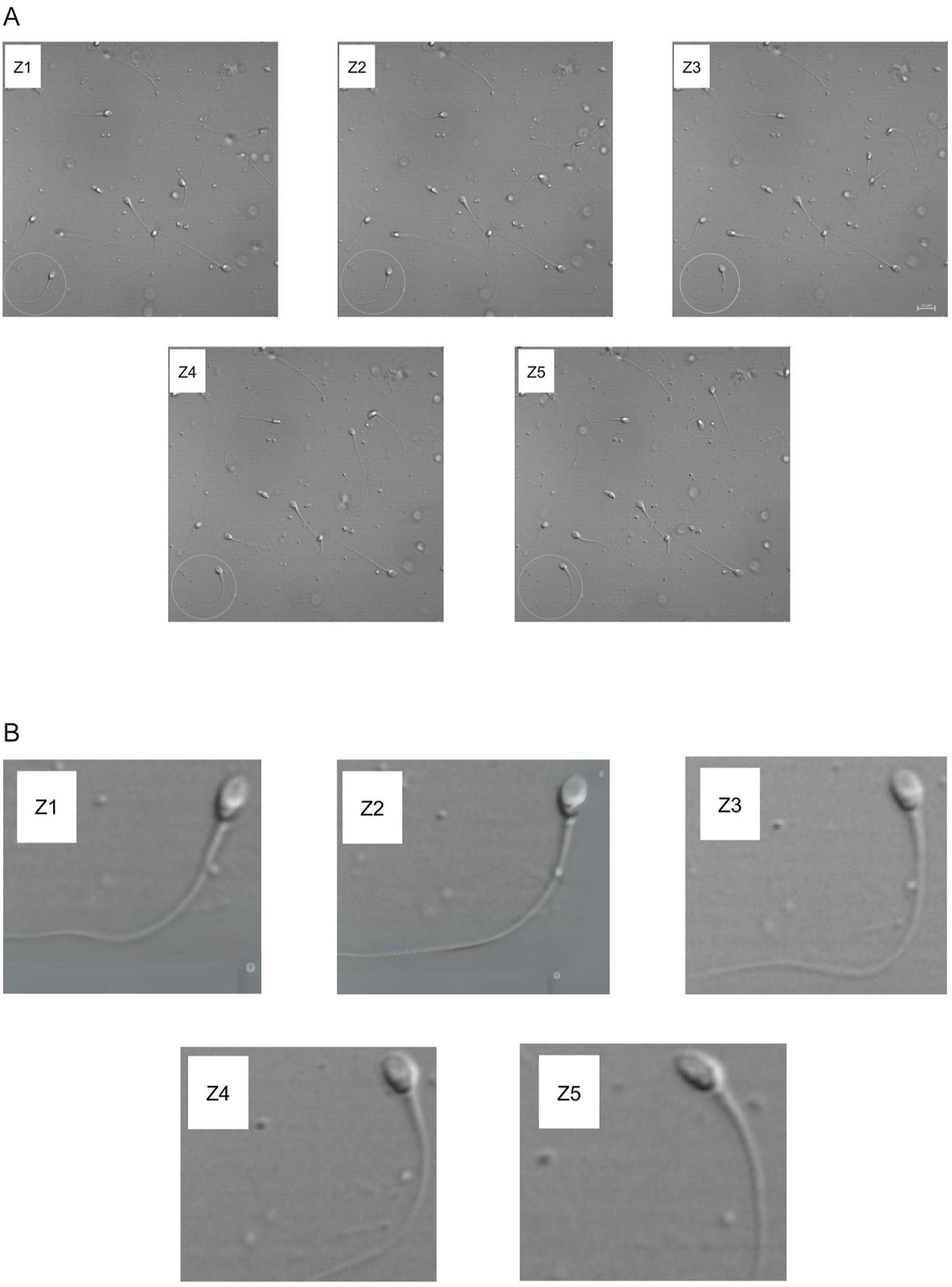

Figure 1

Images captured using a confocal laser scanning microscope (LSM 800). (A) Images captured using a confocal laser scanning microscope (LSM 800) at 40× magnification in the confocal mode (LSM, Z-stack). (Alt-text: series of images captured with a confocal laser scanning microscope at 40× magnification, showing a Z-stack of images in confocal mode. The images provide detailed views of the specimen's structure). (B) Five zoomed-in frames of sperm with normal morphology. (Alt-text: five close-up images of sperm with normal morphology, displaying their typical structure, including the head, midpiece and tail. The frames emphasise the standard appearance of these sperm features).

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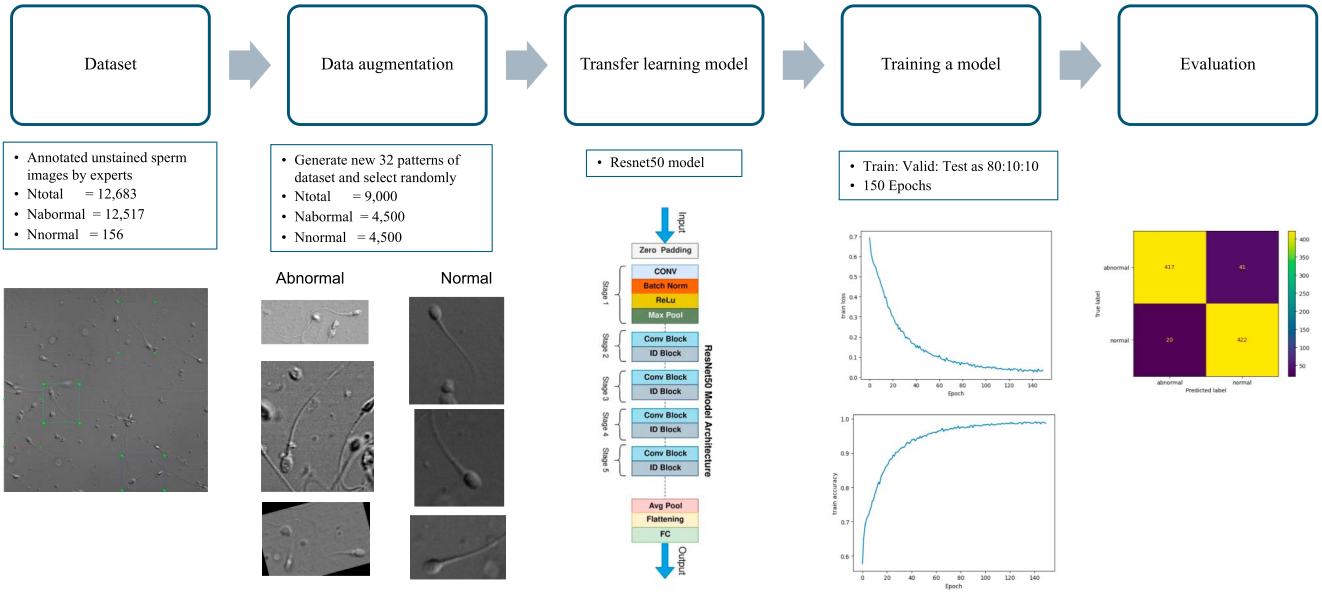

Figure 2

Flowchart showing the development of the AI model for sperm morphology assessment. The flowchart outlines the process of developing an AI model for sperm morphology assessment, from dataset creation to model evaluation. Abbreviations: AI, artificial intelligence. (Alt-text: flowchart depicting the development process of an AI model for assessing sperm morphology. The chart includes steps from dataset creation, data pre-processing, model training, model evaluation and feature elements such as data labelling, feature extraction, algorithm selection and performance validation).

amendments. This study was approved by the Institutional Ethics Committee of the Faculty of Medicine, Prince of Songkla University, on November 28, 2023 (approval no. PSU104.2435172/67-03524). All participants received detailed information regarding the study and provided written informed consent. The study adhered to the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines.

Results

Patient characteristics and semen analysis

Table 1 presents the baseline characteristics and initial semen analysis findings for the study participants. Thirty participants with no underlying diseases and a mean (±SD) age of 30.67 (±5.07) years were enrolled. The average abstinence period before semen sample collection was 2.67 (±0.61) days.

With regard to semen characteristics, the mean semen volume was 2.92 (±1.32) mL. The median (IQR) sperm concentration was 41.99 × 10^6/mL (29.01–78.78 × 10^6/mL). The median total motility rate was 61.66% (45.0–76.83%), while the median progressive motility rate was 51.45% (33.53–66.2%). No active infection or inflammation was detected in any of the samples.

Sperm morphology

The median (IQR) percentages of sperm with normal morphology detected using the three methods were 7.65% (5.95–12.6%), 1.5% (0.63–4.5%) and 8.0% (5.25–10.0%) for the AI model, IVOS CASA and CSA, respectively, with significant between-group differences ($P < 0.001$ for in-house AI vs CASA, $P = 0.02$ for in-house AI vs CSA, and $P = 0.001$ for CSA vs CASA).

Table 1 Baseline characteristics. Data are presented as the mean ± SD, *n* (%) or as the median (IQR).

Parameter	Values
n	30
Age (years)	30.67 ± 5.07
Underlying disease	
No	30 (100%)
Yes	0
Number of children	0.6 ± 0.86
Abstinence (days)	2.67 ± 0.61
Volume (mL)	2.92 ± 1.32
Semen parameter	
Concentration (×10^6/mL)	41.99 (29.01–78.78)
Total motility (%)	61.66 (45.0–76.83)
Progressive motility (%)	51.45 (33.53–66.2)

SD, standard deviation; IQR, interquartile range.

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The in-house AI model and CASA showed the highest correlation coefficient (0.88; Fig. 3A), indicating a strong positive correlation, followed by in-house AI and CSA (0.76; Fig. 3B) and CSA and CASA (0.57); all correlations were significant ($P < 0.05$). Furthermore, both the in-house AI model and CSA showed significantly higher incidence rates of sperm with normal morphology than did the CASA method, with incidence rate ratios of 3.31 and 3.1, respectively. The results were significant ($P < 0.001$), indicating that the observed differences were unlikely to be due to chance. The narrow confidence intervals for both methods suggest that the estimates were precise and reliable.

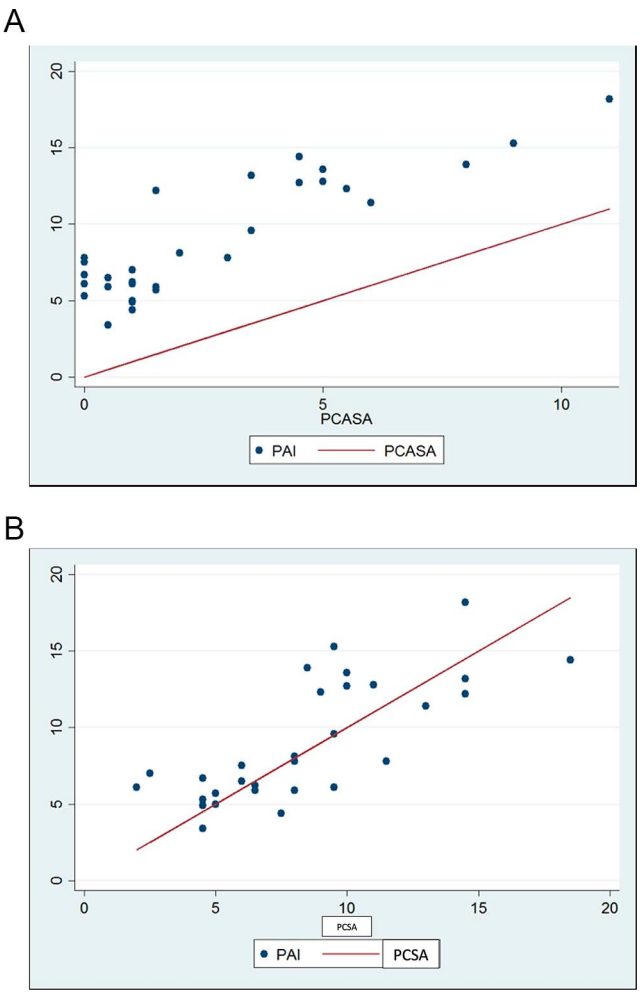

Figure 3

Correlations among the in-house AI, CASA and CSA methods with regard to the detected percentage of sperm with normal morphology. (A) Correlation between the in-house AI and CASA methods. The correlation was determined using pairwise correlations. Statistical significance was set at $P < 0.05$. The plot includes pairwise correlations and indicates statistical significance set at $P < 0.05$). (B) Correlation between the in-house AI and CSA methods. The correlation was determined using pairwise correlations. Statistical significance was set at $P < 0.05$. The plot includes pairwise correlations and indicates statistical significance set at $P < 0.05$). AI, artificial intelligence; CASA, computer-assisted semen analysis; CSA, conventional semen analysis; PAI, percentage of sperm with normal morphology detected by AI; PCSA, percentage of sperm with normal morphology detected by CSA.

Discussion

In the present study, we evaluated live sperm morphology using an in-house AI model without staining and compared the findings with those of CASA and CSA assessments of fixed sperm. The results indicated that the in-house AI model enhanced the accuracy and reliability of live sperm morphology assessments. These findings align with those of a previous study comparing AI optical microscopic technology in LenHooks® X1 PRO with IVOS CASA and CSA for normal sperm morphology evaluation, which revealed significant differences among the three methods (Agarwal *et al.* 2021). A systematic review also reported similar differences between CASA and conventional methods for sperm morphology assessment (Finelli *et al.* 2021). The in-house AI method showed a stronger correlation with the IVOS CASA method than with the CSA method. However, it more frequently reported normal morphological values than did the CASA method. This suggests that assessment of unstained live sperm morphology may require criteria that are different from Kruger's strict standards (Menkveld *et al.* 1990).

Our dataset included 21,600 images with 12,683 annotated unstained sperm images, providing robust training for our machine learning model. To the best of our knowledge, this is the largest dataset, followed by VISEM (Thambawita *et al.* 2023) and SVIA (Chen *et al.* 2022). Furthermore, the application of confocal laser scanning microscopy provided a higher resolution than that provided for previous datasets, facilitating detailed assessment of cellular structures (Elliott 2020). We created a new high-resolution dataset of unstained sperm under low magnification and hypothesised that visualisation of subcellular sperm components is crucial for accurate morphological measurement, as these structures are often indiscernible with bright-field imaging. A Z-stack approach was used to accurately capture sperm head dimensions during movement and noninvasively detect subcellular features, including vacuoles.

The sperm image was annotated with bounding boxes using algorithms adapted from the WHO Laboratory Manual for Human Semen Analysis (sixth edition) (World Health Organization 2021). Currently, there is no standard method for assessing the morphology of unstained sperm under low magnification. The nine datasets classified sperm into normal, abnormal head (tapered, amorphous, pyriform or round), visible vacuoles, abnormal neck and abnormal tail categories. High-resolution images enabled the detection of previously undetectable vacuoles in sperm heads within the SVIA and VISEM tracking datasets.

Our model demonstrated high accuracy in the detection of abnormal sperm morphology, consistent with previous findings (Thirumalaraju *et al.* 2018) of studies that used AI to evaluate the morphology of stained fixed sperm, including the head, midpiece and tail. When tested with the modified HSMA dataset, the model achieved an accuracy of 0.97, indicating its adaptability to lower-resolution datasets while maintaining precision. The average testing time was approximately 0.0056 s per image. These results underscore the model's high accuracy, efficiency and speed in assessing the morphology of unstained live sperm.

Sperm competence, including normal morphology and progressive motility, is linked to successful fertilisation and pregnancy (Guzick *et al.* 2001). Guzick *et al.* found a strong correlation between sperm morphology and *in vitro* fertilisation (IVF) outcomes, noting that lower rates of normal sperm morphology correlate with lower pregnancy rates; however, this was not observed for intracytoplasmic sperm injection (ICSI) (Del Giudice *et al.* 2022). Sperm selection in ICSI relies on subjective morphological evaluation by experienced embryologists using low magnification, which cannot detect DNA damage or assess subcellular organelles. Advanced techniques such as IMSI use high magnification to provide a detailed assessment of sperm morphology and organelles; however, they are time-consuming, expensive and complex (Itoi *et al.* 2022). Therefore, significant expertise is required to effectively select high-quality live sperms for ICSI using IMSI. Conversely, AI and machine learning models offer efficient solutions for evaluating sperm morphology. The proposed AI model in this study could help detect normal sperm in clinical settings, streamline the sperm selection process for ICSI, reduce the workload of embryologists and minimise subjectivity.

A study using an in-house AI model showed high accuracy, reduced assessment time and moderate correlation with CSA for evaluating normal sperm morphology; this indicated the potential of AI to assist in sperm selection during infertility treatment. Accurate sperm morphology assessment is crucial for ART success. The AI model provides a reliable, operator-independent method, reducing the variability and subjectivity of manual analysis, allowing high-quality sperm selection and improving clinical outcomes.

Our approach to the evaluation of live sperm morphology differs significantly from conventional methods, which can only analyse morphology using stain-fixed sperm, which cannot be reused for IVF. In contrast, live sperm analysed using our AI model can still be used in IVF procedures. However, despite the promising benefits of the AI model, further research is needed to refine its algorithms and improve accuracy. Expanding the dataset to include a broader range of unstained live sperm images with subtle morphological abnormalities could enhance the robustness of the model. Clinical trials are essential to validate the efficacy of AI in diverse settings. Future studies should integrate AI with complex sperm selection methods such as microfluidic sperm sorting (Phairatana *et al.* 2023) to improve ART sperm selection efficacy.

In conclusion, our in-house AI model for assessing the normal morphology of unstained live sperm showed a strong correlation with CASA for fixed sperm assessment and significantly higher accuracy than that of CSA. Thus, this AI model is a reliable and accurate tool for sperm morphology assessment without staining, potentially offering an effective alternative to conventional methods. Adopting AI in sperm morphology assessment could revolutionise ART, improve clinical outcomes and increase infertility treatment success rates.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

Funding

This work was supported by the Faculty of Medicine, Prince of Songkla University (Grant No: REC. 66-414-12-3). The funding agency was not involved in the study design, data collection, analysis, interpretation of data, writing of the manuscript or the decision to submit this article for publication.

Author contribution statement

JJ helped in conceptualisation, methodology, software, formal analysis, data curation, writing of the review and editing. PM helped with conceptualisation, methodology, formal analysis, investigation, resources, data curation, writing of the original draft and visualisation. MN helped with software, formal analysis and data curation. CC is the corresponding author and helped with conceptualisation, methodology, validation, formal analysis, investigation, resources, data curation, writing of the review, editing, visualisation, supervision, project administration and funding acquisition. All authors have made substantial contributions to the manuscript and take full responsibility for its contents.

Acknowledgements

The authors thank Mrs Suwadee Wirojkarun, an embryologist, and the staff of the SARC for their help with the recruitment of subjects and testing in this study. We would like to acknowledge the Medical Science Research and Innovation Institute, Research and Development Office, Prince of Songkla University, for granting us access to their confocal laser scanning microscope for collecting sperm images to establish a new valuable dataset of unstained sperm images. Finally, we express our gratitude to Alan Frederick Geater, PhD, from the Epidemiology Unit, Prince of Songkla University, for his guidance and support in the epidemiological aspects of our study.

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