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From Manual & CASA Semen Analysis to AI: Predictive Modeling of Leucocytospermia and Antimicrobial Resistance in Male Subfertility

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ABSTRACT

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Male factor fertility contributes to nearly half of all cases of couple subfertility, and semen analysis remains the cornerstone of its initial evaluation. Traditional manual semen analysis, performed according to World Health Organization (WHO) criteria, is inexpensive and widely available but suffers from substantial inter- and intra-observer variability. Computer-Assisted Semen Analysis (CASA) improved objectivity and introduced quantitative kinematic parameters, yet it still requires expert quality control and cannot, by itself, resolve the clinical question of an underlying genital tract infection. Leucocytospermia — an elevated concentration of peroxidase-positive leukocytes in semen — is a common but frequently under-recognized contributor to male sub fertility, acting through oxidative stress, cytokine-mediated inflammation, and direct sperm membrane and DNA damage. Its management is increasingly complicated by rising antimicrobial resistance (AMR) among common urogenital pathogens, which delays effective treatment and prolongs exposure of sperm to an inflammatory seminal environment. This article reviews the evolution from manual to CASA-based semen analysis, summarises the pathophysiology and diagnostic approach to leucocytospermia, and outlines the growing problem of AMR in andrology. Building on this foundation, we propose and illustrate a machine-learning (ML) framework that integrates CASA kinematic data, seminal biochemical markers, clinical variables and microbiological data to (1) predict the presence and severity of leucocytospermia without relying solely on peroxidase staining, and (2) predict the likely antimicrobial susceptibility profile of the causative organism ahead of formal culture results. Representative, illustrative performance figures are presented to demonstrate how such models could be benchmarked, together with a discussion of validation requirements, explainability, and the ethical and practical barriers to clinical adoption. We conclude that AI-augmented andrology has the potential to shorten the diagnostic pathway for infection-associated subfertility and to support antimicrobial stewardship, provided that models are developed and validated prospectively, transparently, and across diverse patient populations.

Introduction

Infertility is defined as where the couples are not able to conceive after one year of regular, unprotected intercourse. It affects more than 80 million couples of reproductive age worldwide, and a male factor is thought to account for about half of all cases. In current study, male fertility is assessed by analyzing by semen sample according to WHO guidelines. This assessment covers macroscopic analysis like volume, viscosity, consistency, and pH as well as microscopic analysis like sperm concentration, motility, morphology, DNA Fragmentation and further test like peroxidase, HOS. Despite decades of clinical use and technical refinement, conventional semen analysis remains highly variable, with relatively limited accuracy and specificity. Inter- and intra-observer variability further affects the results, largely because laboratories do not always adhere fully to WHO recommendations [18]. At the first WHO Laboratory Manual for the Analysis of Human Semen was published, successive editions have refined reference values and standardised methodology, yet manual analysis of motility and morphology was totally dependent on the skill, training and fatigue level of the technologist performing it.

To minimize the human errors in manual semen analysis the Computer-Assisted Semen Analysis (CASA) in the late 20th century, which substituted objective kinematic measurement and digital tracking of sperm trajectories for subjective visual estimate. However, leucocytospermia—the presence of an abnormally high proportion of active leukocytes—is one of the more clinically significant and often ignored findings in semen analysis that CASA is also unable to resolve. Even when standard parameters of semen appear slightly wrong, leucocytospermia is increasing the oxidative stress, cytokine release, and sperm DNA fragmentation—which is affecting the fertilizing potential of sperm cells. Leucocytospermia is intimately associated with genital tract infection and inflammation [19]. Presence of bacterial and leukocyte infiltration of the male genital system, genital tract infection and inflammation, can seriously effect in spermatogenesis process in testes and sperm cycle in the genital tract impairing the male accessory sex gland's ability to function. Depending on the pathogen the damage can include hormonal imbalances, sperm agglutination, inflammation with accessory sex gland dysfunction, decreased sperm functions and disruption of the blood-testis barrier. Pathogens can operate directly by interfering with male

germ cells or indirectly by stimulating leukocytes which release cytokines and reactive oxygen species (ROS) to cause an inflammatory reaction in the genital tract. Patients with varicocele, trauma or certain hazardous lifestyle circumstances such as smoking or heavy alcohol use also have significantly higher seminal leukocyte concentrations [20].

The introduction of Computer-Assisted Semen Analysis (CASA) in the late twentieth century represented a major methodological advance, replacing subjective visual estimation with digitised tracking of sperm trajectories and objective kinematic measurement. CASA does not, however, resolve one of the more clinically consequential and frequently overlooked findings in semen analysis: leucocytospermia, the presence of an abnormally high concentration of activated leukocytes. Leucocytospermia is closely linked to genital tract infection and inflammation, and its downstream effects — oxidative stress, cytokine release, and sperm DNA fragmentation can meaningfully impair fertilising capacity even when standard semen parameters appear only mildly abnormal. [3, 5]

Compounding this problem is the global burden of antimicrobial resistance (AMR), including resistance among organisms relevant to urogenital infection. However, resistance patterns are organism-, region- and time-dependent, and should not be generalized from global surveillance to individual fertility clinics. [8–10] Empirical antibiotic therapy chosen without culture guidance risks treatment failure, prolongation of the inflammatory insult to spermatogenesis, and further selection pressure for resistant organisms. Artificial intelligence (AI) and machine learning (ML) offer a route to integrate the many data streams generated during an andrology work-up CASA kinematics, seminal biochemistry, clinical history and microbiological data into predictive tools that could flag leucocytospermia earlier and anticipate likely resistance patterns before culture results are available. This article reviews the relevant background and proposes a structured framework for developing and validating such models.

Background

Manual Semen Analysis

Manual semen analysis, as codified in the WHO laboratory manuals, assesses volume, pH, liquefaction, sperm concentration, total motility, progressive motility,

vitality and morphology (typically using Kruger strict criteria). It requires only a light microscope, counting chamber and trained personnel, making it accessible even in resource-limited settings. However, external quality assurance programmes have repeatedly demonstrated substantial inter-observer and inter-laboratory variability, particularly for motility grading and morphological assessment, with coefficients of variation that can exceed 20–30% between technologists. Fatigue, subjective thresholds for borderline motility grades, and differences in staining and fixation technique all contribute to this variability, which in turn can affect clinical decision-making. [3, 4]

Computer-Assisted Semen Analysis (CASA)

CASA systems capture digitised video microscopy of a semen sample and apply tracking algorithms to reconstruct the trajectory of individual spermatozoa across successive frames. This allows objective, reproducible measurement of kinematic parameters that are inaccessible to manual assessment, including curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity ($LIN = VSL/VCL$), straightness ($STR = VSL/VAP$), wobble ($WOB = VAP/VCL$), amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF), in addition to concentration and motility classification. CASA improves standardisation and throughput and generates a far richer dataset per sample than manual assessment, which is a key enabler for downstream machine-learning analysis. Its limitations include potential misclassification of debris and round cells as spermatozoa, sensitivity to chamber depth and sample dilution, the continued need for calibration and internal quality control, and equipment cost, which can limit adoption in lower-resource laboratories. [3, 5]

Leucocytospermia: Definition, Pathophysiology and Diagnosis

The WHO laboratory manual describes a threshold of approximately 1×10^6 peroxidase-positive leukocytes/mL for leukocytospermia; the biological and clinical interpretation of this threshold should be made in context. [3,4] Because round cells in semen may represent either leukocytes or immature germ cells that are morphologically similar on routine staining, accurate diagnosis requires a peroxidase (Endtz) test or, preferably, immunocytochemical staining for the

common leukocyte antigen CD45. Reported prevalence among men attending subfertility clinics ranges from roughly 10% to over 20%, depending on the population studied and the diagnostic threshold applied.

Activated leukocytes, predominantly neutrophils, generate substantial quantities of reactive oxygen species (ROS) as part of the normal immune response to infection or inflammation. In excess, ROS overwhelm the limited antioxidant capacity of seminal plasma and spermatozoa, producing lipid peroxidation of the sperm plasma membrane, impaired axonemal function and reduced motility, and oxidative damage to nuclear and mitochondrial sperm DNA. Pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF- α), along with proteolytic enzymes such as elastase, are frequently elevated in the seminal plasma of men with leucocytospermia and correlate with impaired semen parameters. Recognised causes include bacterial or, less commonly, viral genital tract infection (epididymitis, prostatitis, urethritis), sexually transmitted infections, varicocele, autoimmune processes, and lifestyle factors such as smoking and excessive alcohol use. Diagnostic work-up typically combines semen culture and susceptibility testing, nucleic acid amplification testing (PCR) for atypical or fastidious organisms, urinalysis, and, where indicated, transrectal ultrasound or other imaging. [4, 5]

Antimicrobial Resistance in Male Genital Tract Infections

AMR is an important challenge for infectious-disease management, but evidence specifically linking resistance phenotypes to leukocytospermia in male subfertility remains limited. [8–10] Fluoroquinolone resistance among uropathogenic *E. coli*, extended-spectrum beta-lactamase production among Enterobacteriaceae, macrolide and tetracycline resistance among *Ureaplasma* and *Mycoplasma* species, and the well-documented multidrug resistance of *Neisseria gonorrhoeae* — listed by the WHO among priority pathogens for research and development of new antimicrobials — mean that empirical antibiotic selection is increasingly unreliable. Conventional culture and susceptibility testing typically requires 48–72 hours or longer for fastidious organisms, during which patients are commonly started on empirical therapy that may prove ineffective. Treatment failure not only prolongs the inflammatory insult to spermatogenesis

but also contributes to further selection of resistant strains, creating a cycle that is difficult to break without better diagnostic and decision-support tools.

Rationale for Integrating Artificial Intelligence

A complete andrology work-up can generate a high-dimensional dataset comprising semen parameters, CASA measurements, clinical variables and microbiological findings. Recent AI literature in semen analysis supports the feasibility of analysing such multidimensional data, although clinical validation remains limited. [5–7] dozens of CASA kinematic variables, leukocyte and round-cell counts, seminal biochemical markers, clinical and demographic variables, and, where available, culture and antibiogram results. The relationships among these variables are almost certainly non-linear and interactive — for example, the effect of a given leukocyte concentration on motility may depend on antioxidant capacity, infection chronicity and organism type — and are therefore difficult to capture fully with traditional univariate or simple multivariate statistics. Machine-learning methods are well suited to this type of problem because they can model complex, non-linear interactions among many correlated variables and can be evaluated systematically for predictive performance rather than relying solely on statistical significance.

We propose two complementary predictive tasks. The first is a leucocytospermia classifier that uses CASA kinematic parameters, standard semen parameters and clinical variables to estimate the probability that peroxidase-positive leukocyte concentration exceeds the WHO threshold, potentially reducing reliance on peroxidase staining in laboratories where it is not routinely available. The second is an antimicrobial resistance predictor that uses clinical, demographic and laboratory features available at presentation to estimate the likelihood of resistance to specific antibiotic classes, providing decision support for empirical therapy while formal culture results are pending. [3, 6, 13]

Materials and Methods

Study Design and Population

A staged approach is proposed. The staged approach starts with an cross-sectional cohort of men who attend an andrology or fertility clinic to be evaluated for

subfertility. After this part the staged approach moves to a prospective multicentre validation. The inclusion criteria suggested for the staged approach are that the men must be aged 18–55 years and must undergo semen analysis as part of a subfertility work-up. The exclusion criteria suggested for the staged approach are that the men must not have used antibiotics recently within the 3 months and must not have known azoospermia (absence of sperm cell) that is unrelated, to infection or inflammation. The exclusion criteria also state that the men must not have clinical records. The sample size calculation should give power for the minority class, which may be leucocytospermia-positive or resistance-positive samples. The class-imbalance methods should be applied as needed as described in Section 4.4[13]

Semen Analysis Protocol

- Manual semen analysis performed according to the current WHO laboratory manual (volume, concentration, motility, morphology, vitality, DNA fragmentation). [3]
- Parallel CASA analysis using a validated system, recording concentration, motility categories, VCL, VSL, VAP, LIN, STR, WOB, ALH and BCF. [3, 5]
- Peroxidase (Endtz) test or CD45 immunocytochemistry to quantify true leukocyte concentration and establish the reference-standard leucocytospermia label. [3, 4]
- Semen culture with quantitative colony counts and standard antimicrobial susceptibility testing (disk diffusion or minimum inhibitory concentration methods), supplemented by PCR panels for *E. coli*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma* spp. and *Mycoplasma* spp. [3, 8, 9]

Data Collection and Feature Engineering

Candidate features fall into four domains: (i) demographic and lifestyle variables (age, body mass index, smoking status, alcohol use, occupational heat exposure); (ii) clinical variables (duration of subfertility, prior genital tract infection, varicocele, prior antibiotic exposure); (iii) semen and CASA-derived variables (all parameters listed in Section 4.2, plus derived ratios and variability metrics); and (iv) seminal biochemical markers where available (elastase, IL-6, IL-8, reactive oxygen species assays). Continuous variables are standardised, missing data addressed through multiple

imputation, and categorical variables one-hot encoded. Class imbalance in the outcome labels can be addressed using techniques such as SMOTE (Synthetic Minority Oversampling Technique) or class-weighted loss functions. [6, 8, 13]

Model Development

A tiered modelling strategy is recommended, beginning with interpretable baseline models and progressing to more complex architectures only where they provide a meaningful performance gain: [6, 13]

- Logistic regression with regularisation (LASSO/ridge) as an interpretable baseline.
- Tree-based ensemble methods — Random Forest and gradient-boosted trees (XGBoost/LightGBM) — which typically perform well on tabular clinical and CASA data and provide built-in feature importance. [6, 7]
- Support vector machines as an alternative non-linear classifier for smaller sample sizes.
- Artificial neural networks, and convolutional neural networks applied directly to CASA video/trajectory data, where sample size and computational resources permit.

Model interpretability is prioritised using post-hoc explainability methods such as SHAP (SHapley Additive exPlanations), which assign feature-attribution values to individual predictions. [12] The specific features driving a model should be reported only after analysis of the study dataset.

Model Validation

Model development must use internal testing and if possible separate external testing. Reporting needs to follow the guidelines for prediction models like TRIPOD+AI, which is used for both regression models and machine-learning models. [13] The suggested performance measures are sensitivity, specificity, negative predictive values, area under the receiver operating characteristic curve (AUC-ROC) F1 score and calibration plots shown separately for the leucocytospermia classifier and, for every antimicrobial resistance prediction target.

Statistical Analysis

In addition to model performance metrics I believe it is essential to examine correlations between leukocyte

concentration and CASA kinematic parameters. For example a Spearman correlation between leukocyte count and progressive motility or VSL can provide valuable insight. Direct comparison of manual versus CASA versus AI-augmented performance should also be undertaken and appropriate statistical tests such as DeLong's test, for comparing AUCs should be used to determine significance. [13]

Results and Discussion

The original draft contained illustrative numerical results. Because these values are not derived from a completed analysis, they should not be presented as research findings.

For a submission-ready manuscript, this section should be replaced with results generated from the study dataset and reported according to the prespecified analysis plan. [13].

The biological literature supports associations between seminal leukocytes, oxidative stress and altered sperm function, but the relative importance of individual CASA variables must be established empirically in the proposed dataset rather than assumed in advance. [4, 5]

The trajectory from manual to CASA-based semen analysis illustrates a broader trend in andrology toward objective, quantitative, data-rich diagnostics. Leucocytospermia sits at the intersection of andrology and infectious disease, and its clinical impact is mediated substantially through oxidative stress pathways that are already partially captured by CASA kinematic parameters, even though CASA alone cannot establish the diagnosis. This creates a natural opportunity for AI: models that combine CASA output with a small number of additional clinical and biochemical variables may approach the diagnostic performance of peroxidase staining without requiring it for every sample, which could be particularly valuable in laboratories where peroxidase testing is not routinely performed. [3, 5, 6]

For antimicrobial resistance, the clinical value of a predictive model lies less in replacing culture and susceptibility testing — which remains the diagnostic gold standard — than in supporting the choice of empirical therapy while culture results are pending, and in flagging patients at higher risk of a resistant organism for closer follow-up or earlier escalation.

Table.1 Illustrative comparison of semen parameters by leucocytospermia status

Parameter	Leucocytospermia-negative (illustrative)	Leucocytospermia-positive (illustrative)
Sperm concentration ($\times 10^6/\text{mL}$)	58.4 ± 21.3	41.2 ± 18.7
Progressive motility (%)	46.1 ± 10.2	31.5 ± 11.8
VSL ($\mu\text{m/s}$)	28.6 ± 6.1	20.3 ± 5.9
VCL ($\mu\text{m/s}$)	62.7 ± 9.4	51.8 ± 10.1
Normal morphology (%)	6.8 ± 2.4	4.1 ± 2.0
Seminal IL-6 (pg/mL)	18.2 ± 7.6	54.9 ± 22.3

Values are illustrative mean $\pm$ SD, representative of directionally consistent findings reported in the literature; not primary study data. [4, 5]

Table.2 Illustrative predictive performance for a leucocytospermia classifier

Model	AUC-ROC	Sensitivity	Specificity
Logistic regression (baseline)	0.78	0.71	0.75
Random Forest	0.87	0.82	0.84
XGBoost	0.89	0.85	0.86
Neural network (CASA trajectory-based)	0.90	0.86	0.87

Illustrative figures for framework demonstration only; actual performance must be established through the validation process in Section 4.5.

Table.3 Illustrative antimicrobial resistance patterns among common isolates

Organism	Fluoroquinolone resistance	Macrolide resistance	Beta-lactam resistance
<i>Escherichia coli</i>	28%	N/A	22%
<i>Enterococcus faecalis</i>	18%	N/A	N/A
<i>Ureaplasma urealyticum</i>	N/A	24%	N/A
<i>Neisseria gonorrhoeae</i>	35%	12%	8% (cephalosporin)

Illustrative resistance percentages for demonstration only; real surveillance data are strongly geography- and time-dependent and should be sourced from local antibiograms. [8–10]

Because resistance patterns are strongly influenced by local antimicrobial use and epidemiology, any such model would need to be trained and, ideally, retrained on local or regional surveillance data rather than applied unmodified across settings. [8–10]. Clinical adoption of AI tools in andrology raises several considerations beyond raw predictive accuracy. Explainability is essential: clinicians are unlikely to act on a resistance or leucocytospermia prediction they cannot interrogate, which is why SHAP-based or similar interpretable outputs are recommended throughout Section 4.4. Data governance and patient privacy must be addressed given the sensitive nature of fertility and sexual health data.

Algorithmic bias is a further concern; models trained predominantly on one population may generalise poorly to others, underscoring the need for diverse training data and external validation (Section 4.5). Finally, any AI decision-support tool should be positioned as an adjunct to, not a replacement for, clinical judgement, culture-based confirmation, and existing WHO-based semen analysis standards. [12, 13]

Limitations

- The illustrative data presented in Section 5 are for framework demonstration only and do not represent

findings from a completed clinical study.

- Retrospective single-centre datasets, if used for initial model development, carry a high risk of selection bias and limited generalisability. [13]
- CASA systems vary between manufacturers and settings; kinematic parameter values are not always directly comparable across platforms without harmonisation or site-specific calibration. [3, 6]
- Antimicrobial resistance patterns are highly dependent on local epidemiology and change over time, requiring periodic model retraining and local validation rather than a single fixed global model. [8, 9]
- Cost and infrastructure requirements for CASA and computational resources may limit near-term adoption in low-resource settings, which are often those with the highest burden of untreated genital tract infection.

Future Directions

- Prospective, multicentre studies to develop and externally validate leucocytospermia and AMR prediction models using the framework outlined above. [13]
- Integration of seminal microbiome sequencing and proteomic/metabolomic data alongside CASA and clinical variables. [6, 7]
- Federated or privacy-preserving learning approaches that allow multiple centres to contribute to shared AMR surveillance models without centralising sensitive patient data. [13]
- Real-time, CASA-integrated decision-support software that flags high-probability leucocytospermia cases at the point of testing and suggests empirical therapy options pending culture. [8–10]
- Prospective clinical trials assessing whether AI-guided antimicrobial stewardship in this context improves fertility outcomes and reduces inappropriate antibiotic use. [13]

In conclusion, the evolution of semen analysis from manual microscopy to CASA has progressively increased the objectivity and richness of andrology data, but has not, on its own, resolved the clinical challenge posed by leucocytospermia and the antimicrobial resistance of its underlying causative organisms. Machine learning offers a structured way to integrate CASA kinematics, seminal biochemistry, clinical history and microbiological data into predictive tools that could support earlier recognition of infection-associated subfertility and more rational, stewardship-conscious antibiotic selection.

Realising this potential will require prospective, multicentre, methodologically rigorous studies — along with careful attention to interpretability, bias, and local validation — before such tools can be responsibly incorporated into routine andrology practice. [3, 5, 8, 13].

Author's Contribution

Suman Sharma: Equal contribution of collecting data, analysis, writing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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