Delayed sperm concentration analysis for patient management in remote settings

Karina Evans[1], Dr David Sinclair[1], Dr Doug Johnson[2], Dr Michael Carroll[3]

1. Cytology and Andrology Department, Cellular Pathology, Queen Alexandra Hospital, Portsmouth University Hospitals NHS Trust, Portsmouth, PO6 3LY
2. King Edward VII Memorial Hospital, Stanley, Falkland Islands, FIQQ 1ZZ
3. Department of Life Science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, M15 6BX

Keywords: Testicular microlithiasis, semen analysis, risk stratification, sperm concentration, andrology, healthcare

1. Introduction

Testicular microlithiasis (TM) is a rare condition characterised by multiple microcalcifications within the seminiferous tubules. While often asymptomatic, TM has been associated with an increased risk of testicular malignancy, particularly in individuals with additional risk factors such as infertility or cryptorchidism[1].

Risk stratification relies on clinical examination, tumour markers, and assessment of spermatogenic function. In remote settings, limited access to specialist andrology services creates challenges in delivering guideline-based care. Time-delayed semen analysis offers a practical solution to support the risk stratification and management of TM in these contexts.

2. Aim

To assess the feasibility of time-delayed semen analysis for evaluating spermatogenic function and supporting risk stratification in the management of testicular microlithiasis in remote healthcare settings.

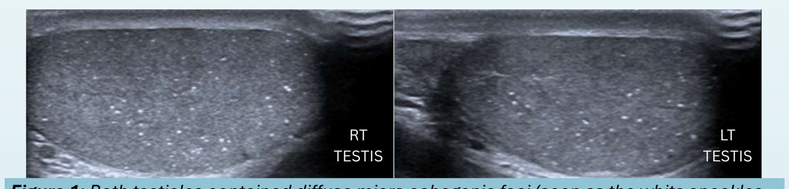

Figure 1: *Both testicles contained diffuse micro echogenic foci (seen as the white speckles in the images). Ultrasound images from KEMH Radiography Department.*

3. Case Presentation

A 20-year-old male presented to King Edward Memorial Hospital, Stanley, Falkland Islands, with longstanding intermittent left testicular pain. He had no urinary symptoms, erectile difficulties, children, or family history of testicular cancer.

Physical examination was unremarkable, with normal testicular size, position, and consistency. Ultrasound confirmed diffuse bilateral testicular microlithiasis with normal colour flow and no hydroceles or cysts present (Figure 1). Serum tumour markers α-fetoprotein and β-human chorionic gonadotropin were within normal limits.

To guide risk stratification and management, assessment of spermatogenic function was required.

Due to the absence of on-site andrology services in the Falkland Islands, Portsmouth Hospitals University NHS Trust (PHUT) was contacted to explore the feasibility of time-delayed semen analysis for assessing sperm concentration, facilitating the transport of a semen sample for evaluation despite geographical constraints.

4. Method

Validation of Time Delayed Sperm Assessment

Six diagnostic semen samples, with documented patient consent, were included in the validation study. Each sample was processed in accordance with the WHO 6th edition (2021) guidelines[2].

An appropriate dilution was prepared using 50 µL of semen measured with a positive-displacement pipette. The diluted aliquots were vortexed thoroughly before 10 µL was loaded into each chamber of an Improved Neubauer haemocytometer (INH) using an air-displacement pipette. The loaded INH was placed in a wet chamber for 20 minutes before being assessed immediately by a biomedical andrologist.

Two counts, comprising a minimum of 200 sperm, were performed, and a dilution factor was applied to calculate the sperm concentration per mL and per ejaculate.

Following this initial assessment, the remaining semen was stored at 2–8°C for seven days. After the storage period, sperm concentration was re-analysed using the same WHO methodology and counting process.

Initial and delayed measurements were then statistically compared using a paired t-test to determine whether storage time had any effect on sperm concentration.

Performing the Time Delayed Semen Analysis

The semen sample was produced in the Falkland Islands into a toxicity-tested, pre-weighed vial.

→ It was couriered via the RAF Airbridge from the Falkland Islands to PHUT via RAF Brize Norton.

→ The sample was received and processed immediately according to WHO (2021) guidelines[2].

→ A report, incorporating the patient's clinical history, was authorised by the Consultant Clinical Scientist.

5. Results

Validation Results

Sperm concentration remained consistent after seven days of refrigerated storage, with no significant differences observed (*p*=0.800)(Figure 2).

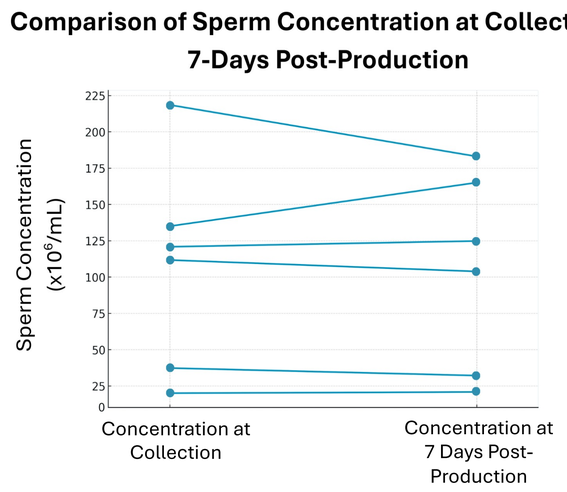

Figure 2: *Paired data plot showing sperm concentration per mL at collection and after seven days of refrigerated storage (n = 6). No significant difference was observed between time points (t(5) = 0.266, p = 0.800, 95% CI [-19.89, 24.49]), indicating stability of concentration during storage.*

Patient Test Results

Time from production to analysis was 56 hours and 42 minutes. Analysis showed a normal volume (2.7 mL) and pH (7.8), with sperm concentration (44.3 ×10^6/mL) and total count (120.4 ×10^6) well above the WHO lower reference limits[2], indicating normal spermatogenic function.

6. Management & Outcome

Diagnosis → **Education**

The patient was diagnosed with diffuse bilateral TM, normal tumour markers, and spermatogenic function. He was classified low risk and managed conservatively.

He received education on regular testicular self-examination (TSE) and was reviewed after six months.

Follow Up ← **Review**

The patient declined an on-site semen analysis but several months later fathered a healthy baby.

As no progression was seen, he was discharged with ongoing TSE advice and rapid re-referral if needed.

7. Discussion

Sperm concentration remained stable after seven days of refrigeration, suggesting that delayed analysis can provide reliable information on spermatogenic function where immediate processing is not feasible. This is particularly valuable in remote settings where transporting samples in hazardous fixatives, such as formalin, is not possible.

There is limited research on the effects of delayed ejaculation-to-analysis intervals on sperm concentration. Historically, postal semen samples were permitted for post-vasectomy testing[3] but were later withdrawn due to concerns about measurement uncertainty and lack of validation[4].

A significant limitation of this study is the small sample size (n = 6), which restricts statistical power and generalisability. In addition, factors such as counting variation, temperature fluctuations, measurement uncertainty and logistical variability were not fully controlled[5]. Larger, multi-centre studies are needed to confirm these findings and support guideline-based care for patients with TM1,6 and other conditions that may affect spermatogenic function in remote locations.

8. Conclusion

Time-delayed semen analysis can provide a practical, non-invasive method for assessing spermatogenic function in remote settings where immediate testing is not feasible. This approach reduces unnecessary invasive procedures and facilitates guideline-based management. However, larger validation studies are required to confirm reliability and inform future clinical practice.

References

1. Barbonetti, A., Martorella, A., Minaldi, E., D'Andrea, S., Bardhi, D., Castellini, C., Francavilla, F. and Francavilla, S. (2019) 'Testicular cancer in infertile men with and without testicular microlithiasis: A systematic review and meta-analysis of case-control studies', *Frontiers in Endocrinology*, 10, p.164
2. World Health Organization (2021) WHO laboratory manual for the examination and processing of human semen. 6th edn. Geneva: World Health Organization
3. Hancock, P. and McLaughlin, E. (2002) 'British Andrology Society guidelines for the assessment of post vasectomy semen samples (2002)', *Journal of Clinical Pathology*, 55(11), pp. 812–816
4. Hancock, P., Woodward, B.J., Muneer, A. and Kirkman-Brown, J.C. (2016) '2016 Laboratory guidelines for postvasectomy semen analysis: Association of Biomedical Andrologists, the British Andrology Society and the British Association of Urological Surgeons', *Journal of Clinical Pathology*, 69(7), pp. 655–660
5. Tomlinson, M.J. (2016) 'Uncertainty of measurement and clinical value of semen analysis: Has standardisation through professional guidelines helped or hindered progress?', *Andrology*, 4(5), pp. 763–770
6. Richenberg, J., Belfield, J., Ramchandani, P., Rocher, L., Freeman, S., Tsili, A.C., Cuthbert, F., Studniarek, M., Bertolotto, M., Turgut, A.T., Dogra, V. and Derchi, L.E. (2015) 'Testicular microlithiasis imaging and follow-up: Guidelines of the ESUR scrotal imaging subcommittee', *European Radiology*, 25(2), pp. 323–330

Acknowledgements

We thank King Edward Memorial Hospital and Portsmouth Hospitals University NHS Trust for their support, and the patient for consenting to share his case for educational purposes.