

Journal Pre-proof

A Case Report on the Prolonged Viability of Post-Mortem Human Testicular Sperm

Jamie Thomas, Joginder Bidhan, M.Sc., Braian Rene Ledesma, Joshua Bitran, M.D.,
Ranjith Ramasamy, M.D.



PII: S2666-3341(23)00036-3

DOI: <https://doi.org/10.1016/j.xfre.2023.03.005>

Reference: XFRE 299

To appear in: *F&S Reports*

Received Date: 13 February 2023

Revised Date: 7 March 2023

Accepted Date: 13 March 2023

Please cite this article as: Thomas J, Bidhan J, Ledesma BR, Bitran J, Ramasamy R, A Case Report on the Prolonged Viability of Post-Mortem Human Testicular Sperm, *F&S Reports* (2023), doi: <https://doi.org/10.1016/j.xfre.2023.03.005>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2023 The Author(s). Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine.

A Case Report on the Prolonged Viability of Post-Mortem Human Testicular Sperm

Jamie Thomas^a, Joginder Bidhan M.Sc.^a, Braian Rene Ledesma^a, Joshua Bitran M.D.^a, Ranjith Ramasamy M.D.^a

^aDesai Sethi Urology Institute, University of Miami, Miami FL

Corresponding Author

Ranjith Ramasamy, M.D.

Director of Reproductive Urology, Associate Professor

Desai Sethi Urology Institute, Miller School of Medicine, University of Miami

1150 NW 14th St, Miami, FL 33136

Phone: (305) 243-4000

Email: ramasamy@miami.edu

Article Type: Case Report

Funding Statement: This research was funded by a NIH Grant R01 DK130991 and Clinician Scientist Development Grant from the American Cancer Society to Dr. Ranjith Ramasamy

Disclosure Statement: No relevant disclosures

Attestation Statement: Data regarding any of the subjects in this study has not been previously published elsewhere. Data will be made available to the editors of the journal for review or query upon request.

Abstract word count: 144

Manuscript word count: 1,740

Capsule: This is the first study to demonstrate viable or motile sperm at 106 hours post-mortem and may have implications on the timeframe that post-mortem sperm retrieval can be performed successfully.

Abstract

Objective: To report a case of post-mortem sperm retrieval with prolonged viability and motility.

Design: Case Report

Setting: Jackson Health System and Miami-Dade County Medical Examiner Department

Patient: A 44-year-old African American male patient with a history of recreational marijuana use and occasional alcohol consumption, who died from a cardiac arrest due to drug overdose.

Intervention: Multiple testicular biopsies and sperm analyses.

Main Outcome Measures: Sperm viability and motility of testicular biopsies at serial time intervals.

Results: Sperm obtained from the testis in the morgue remained viable and motile even at 106 hours (> 4 days) post-mortem.

Conclusion: Our study found that sperm obtained from the testis remained viable and motile even after being thawed following cryopreservation, even when obtained up to 100 hours post-mortem. This may have implications on the timeframe that post-mortem sperm retrieval can be performed successfully several days after death.

Keywords

Case report, post-mortem sperm retrieval, sperm analysis, biopsy, assisted reproductive technology

Introduction

Post-mortem sperm retrieval (PMSR) is used to collect viable sperm from a recently deceased man for future use in assisted reproductive technology (ART) (1). It has been successfully demonstrated to produce viable offspring and is often used in situations of an unexpected death or when a patient has expressed desire to have children after death (2). The procedure was first described in 1980 by Rothman and remains relatively rare, although requests for PMSR are increasing in frequency (3-5). While some countries such as Israel and the United States approve of PMSR, many question the ethical dilemmas surrounding this procedure (6, 7). Not only does it pose a challenge for the establishment of universally accepted guidelines, but it is also controversial in the military where a U.S. Army judge has argued that PMSR should be limited to those who cryopreserve their sperm prior to death (8, 9). However, as most soldiers do not cryopreserve their sperm prior to deployment, this may be controversial when widows of these soldiers request PMSR (8). According to the Ethics Committee of American Society for Reproductive Medicine, post-mortem reproductive procedures may be considered ethically justifiable under certain circumstances including written consent from the deceased or a request by the surviving spouse or partner (10). Regardless of its controversial nature, the procedure is limited by several factors that affect the viability of post-mortem sperm such as the time between death and collection, comorbidities of the patient, and storage conditions of the sperm (11, 12).

Analyzing the viability and cryopreservation of posthumous sperm provide an opportunity to offer patients and their families greater insight to help inform decisions on whether to pursue this option in specific cases. However, medical literature reports extracting viable sperm within 24-36 hours after death, which may be a challenge in certain circumstances

(13). We report a unique case of a deceased 44-year-old man from a drug overdose in whom we observed sperm viability and motility on testis biopsy analysis even at 106 hours post-mortem.

Case Report

Our patient was a 44-year-old African American male with a history of recreational marijuana use and occasional alcohol consumption was brought to the Jackson Health System in Miami in cardiac arrest. He had reported symptoms of lightheadedness, nausea, and vomiting approximately 30 minutes before arriving at the emergency department. The patient had no significant past medical or surgical history, no relevant family history, and was sexually active with one female partner, using condoms inconsistently.

Upon arrival, the patient was in asystole and received treatment with an automated external defibrillator, intubation, and administration of Amiodarone, Sodium Bicarbonate, and Epinephrine. A single shock was administered for a pulse check with Ventricular Fibrillation; however, the patient was pronounced dead at 10:43 pm, approximately 35 minutes after the onset of cardiac arrest.

The patient was identified by our study team through a search of patient lists from the Miami-Dade County Medical Examiner Department (ME) for male patients aged 15-60 years old who had experienced sudden death, were not hospitalized, and had no comorbidities. A pre-approved Institutional Review Board (IRB) protocol through the University of Miami Health System (UMH) and Jackson Health System was used to establish this study. Our research group was only provided with deidentified subject information from the ME office. If a case met parameters for this study, the responsibility fell on our research group to obtain next-of-kin information from the ME and be the primary point of contact for explaining the premise of the study and consenting the next-of-kin. The next-of-kin consent was approved by a UMH ethics

team, as well as the acting director of the ME office. The ethical guidelines we followed for next-of-kin consenting included competent supervision with appropriate professional decorum. If needed, interpreters were obtained for the purposes of informed consent. We informed next-of-kin that the study would not impact the time needed for the ME to examine the body and transfer it to the desired funeral home as well as confirmed that damage would be kept to a minimum, only involving the scrotum. All questions of the next-of-kin were answered, and consent was not coerced in any way. The next-of-kin was given every opportunity to rescind consent during the study.

The patient's next of kin provided informed consent for post-mortem sperm retrieval and the submission of a case report for publication. The patient's testicle was removed and biopsied at 13 hours post-mortem, and four samples were collected and placed into vials containing sperm wash. The testicle was stored in an airtight, sealed container in a refrigerator at 45°F in the morgue after the initial biopsy since the body had to be returned to the family after post-mortem was completed by the medical examiner. The biopsy site remained open between biopsies, but the exposed tissue was discarded and unexposed tissue was used for subsequent biopsies. Testis biopsy analysis was conducted every 24 hours following the first biopsy to determine the viability of the spermatozoa, and biopsies and analyses were continued until the spermatozoa were no longer viable. Viability was assessed using the eosin alone method according to the 5th edition of the *World Health Organization laboratory manual for the examination and processing of human semen* (14). We utilized a centralized review of biopsy samples with a trained andrology technician, who conducted the testicular sperm analysis for all samples.

Post-mortem sperm retrieval was performed on the patient's testicles by biopsy at 13, 34-, 58-, 82-, and 106-hours post-mortem. Sperm viability was found to be 67%, 57%, 47%, 34%,

and 22% respectively at these intervals (Figure 1), while motility with pentoxifylline was reported at 26%, 19%, 7%, 11%, and 5% at the respective intervals (Figure 2). The last surgically extracted samples contained minced epididymis and vas deferens, and the collected testicular fluid was cryopreserved using the fast-freezing technique with the aid of a cryoprotectant media. Post-thaw analysis was performed 24 hours after the cryopreservation of the last testicular post-mortem sample and the sperm viability and motility with pentoxifylline were 5% and 1% respectively.

Discussion

Although PMSR is a relatively simple procedure, it raises numerous ethical and moral issues (6). Faced with situations in which widows of deceased soldiers request PMSR and others involving the sudden death of a spouse, we sought to identify whether 36 hours post-mortem, the recommended maximum time within which viable sperm should be retrieved, is truly accurate (13). Our case presents a unique example of successful sperm retrieval, as it demonstrates the longest period in which viable and motile post-mortem sperm has been retrieved.

Currently, it is well-established that sperm quality diminishes with time, as evidenced by the decrease in sperm viability and motility observed in our patient at subsequent biopsies and sperm analysis (12). Therefore, it is advisable to perform PMSR as soon as possible after death to maximize the chances of successful sperm retrieval. According to current literature, PMSR should be performed within 36 hours of death (13). However, our case demonstrates that viable sperm can still be retrieved up to 106 hours post-mortem, contradicting this recommendation. Given the small sample sizes in the limited number of reported cases on this topic, it is important to note that these clinical recommendations are subject to change with additional research as new findings become available (15).

A previous study determined there is no association between the cause of death and the likelihood of successful sperm retrieval, and PMSR may be recommended for patients if the fatal event did not damage the reproductive organs (13). However, even if the reproductive organs remain undamaged, the patient's fertility and chances of successful PMSR may already be compromised due to pre-existing comorbidities such as hypertension, diabetes, sexually transmitted infections, tumors, and various other diseases (16, 17). In contrast, our case may have been ideal for PMSR, as the patient had no significant past medical, surgical, or family history, which may explain the ability to retrieve viable sperm over 100 hours post-mortem.

There are several techniques for sperm retrieval in a post-mortem patient, including manual stimulation, electroejaculation, epididymal or testicular sperm aspiration, testicular biopsies, and orchiectomy with epididymectomy (13, 18, 19). In our case, the latter technique was used. Needle biopsy is generally discouraged in PMSR due to the limited amount of sperm obtained through aspiration (20). However, due to the high heterogeneity and lack of comparative studies evaluating the different techniques, it is difficult to determine whether the technique used in this case influenced the success of collecting viable sperm after an extended period of time (18).

The findings presented in this study are based on a single patient and therefore have limited generalizability. Additionally, biopsy samples were obtained from a testicle that was extracted 13 hours after the patient's death and stored in a refrigerator. It is possible that spermatogenesis in this scenario may differ significantly from spermatogenesis in a testicle within the scrotum of a deceased individual. Thus, additional investigations are necessary to compare and analyze the differences between the two situations. Further research is necessary to collect data from a larger sample size to determine the reproducibility of our results. It is possible

that there are confounders in the medical history of our patient that we were unable to account for due to the sudden nature of the patient's death and the limited availability of medical records identifying the etiology of the patient's cardiac arrest. While our data is derived from the sperm collected from a single patient, the observation of viable and motile sperm over 100 hours post-mortem highlights the need for additional studies to explore the potential for extending the current recommended time frame of 36 hours for PMSR.

This patient, who had no significant comorbidities prior to death, presented with viable sperm at 106 hours post-mortem, a significantly longer time frame than what is reported in the literature. The viability of posthumous sperm for reproductive purposes is a topic that may be raised during medical consultations, and the findings of this study have the potential to inform the guidance provided to patients and their spouses on this matter. Although further research is needed to determine the optimal timing and techniques for post-mortem sperm retrieval to maximize the chances of success in assisted reproduction, the data suggests that the window for successfully retrieving viable and motile sperm may be wider than previously documented in medical literature.

Acknowledgements

We would like to thank Dr. Justin Achua for his contributions to starting this project and helping with IRB approval and consent forms.

References

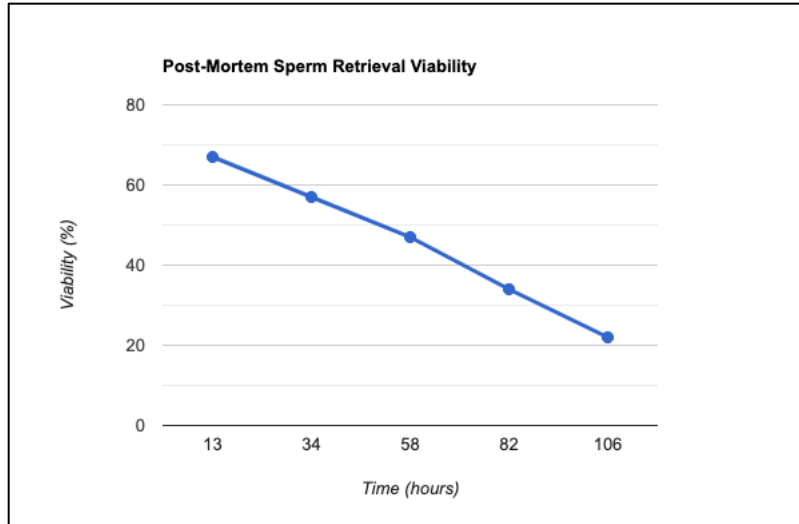
1. Zinkel AR, Ankel FK, Milbank AJ, Casey CI, Sundheim JJ. Postmortem Sperm Retrieval in the Emergency Department: A Case Report and Review of Available Guidelines. *Clin Pract Cases Emerg Med* 2019;3:405-8.
2. Belker AM, Swanson ML, Cook CL, Carrillo AJ, Yoffe SC. Live birth after sperm retrieval from a moribund man. *Fertil Steril* 2001;76:841-3.
3. Hurwitz JM, Macdonald JA, Lifschitz LV, Batzer FR, Caplan A. Posthumous sperm procurement: an update. *Fertil Steril* 2002;78:242.
4. Rothman CM. A method for obtaining viable sperm in the postmortem state. *Fertil Steril* 1980;34:512.
5. Gat I, Umanski A, Kaufman S, Kedem A, Avraham S, Youngster M *et al.* What can we learn about posthumous sperm retrieval after extra long-term follow-up? *J Assist Reprod Genet* 2022;39:1661-5.
6. Strong C, Gingrich JR, Kutteh WH. Ethics of postmortem sperm retrieval: ethics of sperm retrieval after death or persistent vegetative state. *Hum Reprod* 2000;15:739-45.
7. Orr RD, Siegler M. Is posthumous semen retrieval ethically permissible? *J Med Ethics* 2002;28:299-302.
8. Hans JD, Yelland, E.L. American Attitudes in Context: Posthumous Sperm Retrieval and Reproduction. *Journal of Clinical Research Bioethics* 2013;4.
9. Pennings G. Balancing rules in postmortem sperm donation. *J Med Ethics* 2022;48:270-1.
10. Ethics Committee of the American Society for Reproductive Medicine. Electronic address Aao, Ethics Committee of the American Society for Reproductive M. Posthumous retrieval and use of gametes or embryos: an Ethics Committee opinion. *Fertil Steril* 2018;110:45-11.
11. Sharma R, Kattoor AJ, Ghulmiyyah J, Agarwal A. Effect of sperm storage and selection techniques on sperm parameters. *Syst Biol Reprod Med* 2015;61:1-12.
12. Tumram NK, Bardale RV, Ambade VN. Sperm motility and viability extracted from penile tract of corpses: A preliminary study. *Med Leg J* 2016;84:132-4.
13. Shefi S, Raviv G, Eisenberg ML, Weissenberg R, Jalalian L, Levron J *et al.* Posthumous sperm retrieval: analysis of time interval to harvest sperm. *Hum Reprod* 2006;21:2890-3.
14. Organization WH. WHO laboratory manual for the examination and processing of human semen. World Health Organization 2010.
15. Raziel A, Friedler S, Strassburger D, Kaufman S, Umansky A, Ron-El R. Nationwide use of postmortem retrieved sperm in Israel: a follow-up report. *Fertil Steril* 2011;95:2693-5.
16. Guo D, Li S, Behr B, Eisenberg ML. Hypertension and Male Fertility. *World J Mens Health* 2017;35:59-64.
17. Shiraishi K, Matsuyama H. Effects of medical comorbidity on male infertility and comorbidity treatment on spermatogenesis. *Fertil Steril* 2018;110:1006-11 e2.
18. Ovics SO, Baram S, Nothman S, Weiss A, Beck-Fruchter R. Perimortem and postmortem sperm acquisition: review of clinical data. *J Assist Reprod Genet* 2022;39:977-86.
19. Kroon B, Kroon F, Holt S, Wong B, Yazdani A. Post-mortem sperm retrieval in Australasia. *Aust N Z J Obstet Gynaecol* 2012;52:487-90.
20. Jequier AM, Zhang M. Practical problems in the posthumous retrieval of sperm. *Hum Reprod* 2014;29:2615-9.

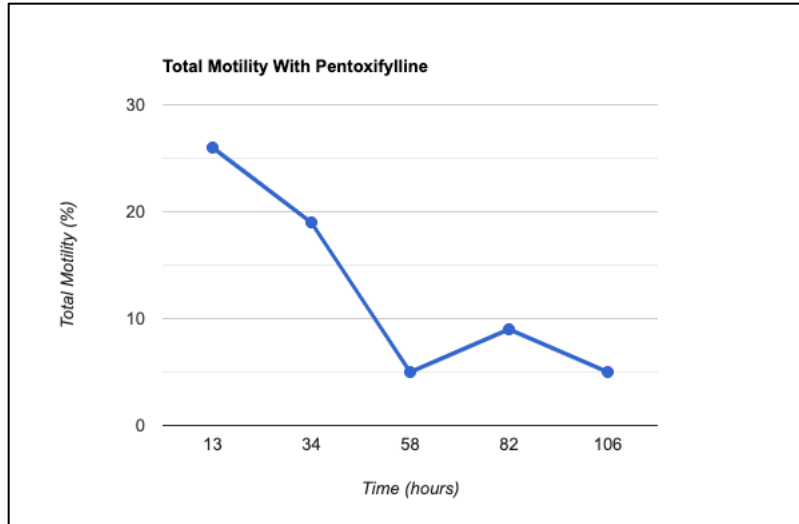
Figure Captions

Figure 1. Post-Mortem Sperm Retrieval Viability. A line graph depicting the decrease in viability of sperm at each biopsy interval.

Figure 2. Total Motility with Pentoxifylline. A line graph depicting a general decrease in total motility of sperm with pentoxifylline at each biopsy interval.

Journal Pre-proof





Journal Pre-proof