

Practical problems in the posthumous retrieval of sperm

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ABSTRACT: This communication discusses the practical problems that arise during the collection and processing of sperm that have been retrieved posthumously. It is based on a small group, namely the last six men from whom we carried out posthumous retrieval. The reason for each retrieval, the method of that retrieval, the assessment of the sperm retrieved, the subsequent viability of the sperm and their storage method are discussed. The many ethical and legal problems that arise both before and after posthumous sperm retrieval are huge in their complexity. Therefore, they will not be discussed here and this communication will be limited to the practical aspects of posthumous sperm retrieval. The purpose of this communication is to make some suggestions that will facilitate such collections. The whole subject of posthumous sperm collection is gaining increasing clinical importance and has begun to interest the media as demonstrated by the recent national coverage in an Australian newspaper.

Key words: posthumous sperm collection

Introduction

The first posthumous sperm collection is reported to have occurred on the field of the Battle of Waterloo after a young woman claimed to have become pregnant using semen obtained from a dead soldier. Although this story is likely to be apocryphal, acute severance of the spinal cord such as that caused by a sword or a bullet is very frequently associated with ejaculation.

Thus in such a circumstance, a young woman might have recognized the presence of semen in the trousers of her loved one and carried out some form of self-insemination. If this story is true, this must be the first case of post-mortem sperm collection.

However, the first authenticated case of posthumous sperm retrieval was reported by Rothman (1980). However, when this sperm collection was performed the patient was comatose and not dead and therefore this sperm collection was not strictly an example of posthumous sperm collection even if the sperm so collected were used after the patient's death.

However, a more recent case of posthumous sperm collection took place in the United Kingdom and involved Mrs Diane Blood whose husband died suddenly and unexpectedly during treatment of the couple's infertility. Sperm were collected posthumously but their use in the generation of a conceptus was forbidden by Human Fertilization and Embryology Authority in the United Kingdom. Finally, Mrs Blood was allowed to export the sperm to Europe where successful treatment was achieved. This case generated considerable interest, both ethically and legally.

It has been known for some time that spermatozoa can remain viable and are even capable of generating a conceptus for 24 h or more after death or under ideal conditions for as long as 36 h after a patient's demise (Benshushan and Schenker, 1998). In this communication, we report the circumstances and results of the sperm collection in the last six males from whom we attempted post-mortem sperm collection.

Using these six men, we devised a method of management that allows us to maximize sperm numbers to avoid storing non-viable sperm and to avoid the collection and processing of the sperm taking place in 'unsocial' hours.

This paper is simply a 'how to do' collection. No reference is made to the ethical or legal aspects of posthumous sperm collection, which have been very fully covered in the past (Bahadur, 2002). It describes the change in thought that we went through as our experience with this procedure matured and the authors hope that this process of maturation will be helpful to others.

Our response to the request for posthumous sperm retrieval

The request to us for the collection of sperm after death usually comes as a phone call from a close relative of the deceased, often his wife or partner. However, in Western Australia we have a problem. In this State, there is an anomaly in the Law as one Act of Parliament says that we can collect sperm while another indicates that we cannot store

such sperm (Human Reproductive Technology Act, 1991). This is, of course, nonsensical as in this context there is no point in collecting sperm if we cannot store them.

In the early days of this study, it was suggested that we get a Supreme Court Order as such an Order overrides the Law and as this legal impasse still exists, the Order not only allows us to proceed with the sperm collection but also provides the clinician with protection from the Law should that ever be a problem. Supreme Court Justices are also available round the clock and their availability may be useful should there be any time pressures on such a sperm collection, although in our experience, such pressure has never been a problem. However, one sad aspect of a Supreme Court Order is its high cost which the family of the deceased will have to pay.

Although few jurisdictions will have to contend with such legal nonsense, our experience of this procedure indicates that it is important that any clinician who takes on this procedure must be fully aware of the legal restrictions in their particular State or jurisdiction since prosecution could result. We do not carry out posthumous sperm production unless a Supreme Court Order is shown to us which indicates that we can go ahead and collect and store sperm. We hope that one day this legal mess is sorted out.

One of the main problems in Western Australia is the thorny issue of consent. The Law in Western Australia is greatly perturbed by the fact that after death consent cannot be given or withdrawn. However, it has been suggested that all male patients when delivering sperm for cryopreservation should sign an 'opt in' consent form indicating that should their death occur prematurely, they can request that any cryopreserved sperm of theirs can be used for generating a pregnancy in their wife or their *de facto* partner. However, for such a process to be value, the patient must have attended some form of clinic and this was the case in only one of the six patients in this study.

In these circumstances, if the local legal and governing bodies are unable to assist, it is important that individual organizations and clinics should at least develop their own institutional policies that are in line with the Law in their individual jurisdiction (Smith and Lipshultz, 2013).

The six patients that underwent posthumous sperm retrieval

This small study is based on the last six cadavers from whom we have been asked to collect sperm posthumously. There were two previous sperm collections but we were at the time so confused by the requisites of the Law that as a consequence, the sperm were collected rather haphazardly. For these reasons, these two cadavers were excluded from this study. It is clear that at least some training is needed for clinicians who are inexperienced in the surgical technique.

Initially, we decided that all retrievals would involve the collection of testicular tissue as this can allow for successful sperm collections even from men with severe primary testicular damage. It must be remembered that usually we will have to collect sperm from men about whose fertility we know nothing and probably never will.

One aspect of posthumous sperm collection is that these cadavers are not your patients. If you are a Gynaecologist or Urologist, your sole job is to collect and process the sperm. Only rarely, will you have a copy of his semen analysis and more rarely still, will there be a history of infertility. Thus, you will know very little about the cadaver and after the sperm retrieval, you will never see him again.

This lack of contact however may have consequences as the cadaver could, particularly if the sperm are few in number, have a variety of genetic causes of his infertility. Such problems have never been discussed in relation to posthumous sperm retrieval. It could well be argued that these anomalies should be sought prior to any use of the retrieved sperm in the generation of a conception. Thus, the sperm retrieval should perhaps be accompanied by a blood sample that could be examined for this and like abnormalities along side the regular blood test for hepatitis and the immunodeficiency virus that is done on all men who have suffered an unnatural death.

We also decided that, to be able to generate a conceptus using these sperm, every female partner would be treated using IVF/ICSI.

It is now time to examine the six men in this study.

Patient 1

The first of these individuals was a man in his mid-20s who died of multiple injuries from a motor vehicle accident. The accident occurred a long way from town and by the time that his body arrived in the City, he had been dead for 4 days and it became clear that putrefaction had begun. Collection of sperm was declined and the situation was explained to the close relatives.

We thought that it would be a waste of time collecting his sperm and that it was better not to spend the large sum of money needed to obtain the Supreme Court Order.

Patient 2

The second case, again a man in his mid-20s who had died of injuries sustained in a motor vehicle accident. This accident also occurred out of town and we discovered on his arrival that he had been dead for ~48 h.

Testis size was normal and we carried out several biopsies of ~5–10 mm in diameter from one testis in which there were plentiful numbers of motile sperm.

Up to this point, we had decided to take single biopsies and thus limited the amount of tissue taken as a mark of respect for the dead man. However, on reflection, we decided that we should be taking as much tissue as possible. After all, if we find a case where there are very few sperm in the testis, we must have enough tissue to provide us with sufficient sperm, not just for one cycle of IVF/ICSI but enough to generate multiple embryos over more than one cycle of treatment. The pregnancy rate from a single cycle of IVF will not necessarily be very good and we will not be getting a second chance at sperm collection in these men. From Patient 2 we collected several large biopsies in which there were plentiful numbers of motile sperm. The idea that the collection of large amounts of tissue was of value was beginning to become important to us. The sperm in these biopsies were cryopreserved.

Patient 3

The third patient was a young man of 19 who died of brain damage as the result of a head injury sustained during an amateur game of football. In this young man, testis size was normal. Two large biopsies were taken from one testis and these contained large numbers of motile sperm.

Patient 4

This 41-year-old man committed suicide by hanging himself. He had been attending an infertility clinic in town and was known to have a low sperm count, but the cause of his oligozoospermia had not been determined.

How long he had been hanging was not known but it was certainly no more than 2–3 h.

That afternoon, the ambient temperature in the city was 41°C. The body first had to be examined fully by the Forensic department and we were unable to collect sperm until the following day. We managed to collect the sperm some 18–20 h after death. On taking a large testicular biopsy, no motility whatsoever was seen in the contained spermatozoa. Despite this finding, we cryopreserved this tissue and some days later we carried out an Eosin Y test and a hyper-osmotic swelling test (Buckett, 2003; Jayendran *et al.*, 1992) on a straw of the cryopreserved but non-motile sperm. Both tests indicated cell death. That day we also tried to stimulate sperm movement using pentoxifylline (Cummins *et al.*, 1991) but no motility was generated. We could therefore assume that all the sperm were dead. In Western Australia, State Law forbids us from using dead sperm in an IVF cycle. Thus, with permission from the next of kin, those sperm that had been collected posthumously were removed from the tank and discarded.

Quite why all the sperm were dead remains unclear. The collection was carried out within 24 h of death and there was certainly some motility in past semen analyses. There was also reasonable numbers of sperm in the large biopsy that we took post-mortem. This problem could have been the result of the ambient temperature that day as a greatly increased temperature will enhance autolysis.

However, this case demonstrates the occasional need for sperm function tests if only to separate dead from living sperm and this is of special importance in Western Australia. It thus became obvious to us that not only large biopsies are needed but also where possible much larger numbers of sperm.

Patient 5

This 27-year-old man died as the result of a head injury following a fall. A crime was suspected here and therefore the Forensic Department spent all day undertaking its investigations. We managed to obtain our testicular tissue some 12 h after death. Testicular size was normal.

This time we excised the contents of the whole of one testis and the tissue contained plentiful numbers of sperm. An amount of tissue that was deemed sufficient for five to six cycles of IVF/ICSI was cryopreserved. As motility was good, no sperm function tests were needed.

With this case we learnt another lesson and that was that we could safely leave the tissue in medium overnight, thus avoiding hours of night work (Jahnukainen and Stukenberg, 2012). The remaining tissue was maintained in medium at 35°C, thus avoiding an 'all night' session and also avoiding work at 'unsocial hours'. Such a window for the maintenance of tissue viability also occurs in ovarian tissue (Dittrich *et al.*, 2012).

Patient 6

A 42-year-old man committed suicide by hanging. On being cut down, he was found to be alive but was severely brain damaged and was also suffering from multiple organ failure. He was put on life support but on the following day was found to be brain dead. His life support was switched off. The right testis was emptied of its contents some 5 h after death. The testicular tissue so obtained contained large numbers of motile sperm. Sufficient tissue to carry out five to six IVF cycles was cryopreserved.

The principle that we now use is that all the contents of one testis can be excised and that sufficient sperm to provide five to six cycles of IVF/

ICSI should be cryopreserved. Having confirmed that the tissue contains plentiful motile sperm, any excess tissue can be discarded. Thus, not all the tissue obtained from one testis need be processed, provided that, the concentrations and motility of the sperm in that tissue is good. What that concentration and motility should be and how it relates to sperm numbers cannot be ascertained by this study.

What this study does show is that one must be prepared to take large amounts of tissue in order to be able to carry out multiple cycles of IVF/ICSI where necessary.

There should also be sufficient sperm to carry out sperm function tests where they are required. There is no point in storing dead sperm in cryotanks and charging the patient for this service.

A recommended method of collecting sperm posthumously

In all six men in this study, an open biopsy was carried out. This allows for the collection of large amounts of tissue and in the last two of these six cadavers in this study the entire contents of one testis was excised. This latter procedure supplied us with a copious amount of tissue and we now know that this can survive in culture overnight so that the processing of this material does not extend into the night or into what is known as 'unsocial' hours.

We intend to continue excising the entire contents of one testis in every case of posthumous sperm retrieval that we are asked to perform in the future and in this tissue, we will cryopreserve sufficient numbers of sperm from which we can carry out approximately five to six cycles of IVF/ICSI. Thus, we aim to cryopreserve multiple straws with ~100 000 sperm in a single straw and to hold one straw for post-thaw assessment of the cryopreserved sperm.

The posthumous retrieval of sperm

The method of collection now always involves the removal of the entire contents of one testis. The scrotum is exposed and the scrotal skin is cleaned with iodine solution in order to render the surface of the skin sterile. As the testicular tissue collection usually takes place in the mortuary, I am sure that absolute sterility is difficult to achieve in that environment. It was interesting to note that in the two straws taken from Patient 4 that were defrosted in order to carry out the sperm function tests, there was no evidence of infection nor was there obvious contamination in the tissue from Patient 5 that had been held in medium overnight.

A longitudinal incision is made down the mid-line of the scrotum and one testis is brought out through the scrotum. A longitudinal incision is now made down the length of the exposed testis and the entire contents of the testis is excised and placed in medium. The medium used was Quinn's Advantage Sperm Freeze (Sage Media Australia). This is a HEPES-buffered salt solution containing the cryo-protectant glycerol in equal volume in the medium.

A number of tubules are now isolated from the main mass of tissue and are teased open using a pair of fine needles while the tissue is being examined microscopically at a magnification of $\times 400$.

In normal tubules, a large number of sperm tails are seen together with free spermatozoa that are present in the lumen of the seminiferous tubule. Having confirmed that sperm are present in large numbers, the tissue is then cut into small pieces and aspirated into cryopreservation

straws which are then sealed, ready to undergo freezing. Tissue that contains sufficient sperm to carry out approximately five to six cycles of IVF/ICSI are prepared in this way.

The process of cryopreservation

The method used for cryopreservation was the standard technique of preserving sperm using the Planar Freezing machine (Yovich and Grudzinkas, 1990) and has been a standard method of freezing sperm for many years. The tissue was first cut up into small pieces and suspended in an approximately equal volume of the Quinn's Advantage Sperm Freeze medium. After collection of the tissue, the seminiferous tubules were teased open to reveal the presence of any free sperm in the tubule. As many as possible of the free sperm are then aspirated, in the medium, into 0.25 ml straws and the end sealed with cement. The straws are then labelled appropriately and are loaded into the Planar Freezing machine (Planar Australia, 360–1.70). This machine is programmed to drop the temperature evenly from room temperature to around -80°C over a period of some 10–14 min. All the straws are then plunged into the Dewar of liquid nitrogen to achieve a storage temperature of -196°C . An appropriate amount of tissue suspended, in medium, and aspirated into larger straws of 2.5 mm in diameter was cryopreserved in the same way.

One straw containing free sperm was then thawed and the post-thaw motility is examined to make sure that a sufficient number of motile and thus viable, sperm survive the freeze. If insufficient sperm survive the freeze, further tissue can be examined and a poor prognosis can be given to the relatives. These techniques allow us to collect sufficient sperm for several cycles of IVF/ICSI and warn the relatives if the sperm function is not good.

HIV and hepatitis B and C

Testing for HIV and hepatitis is routinely carried out in all patients who have suffered an unnatural death. These tests were negative in all the six cadavers described in this paper.

Discussion and conclusions

This report details the six last cases of posthumous sperm retrieval performed by the senior author (A.M.J.) who has a professional life time experience in Clinical Andrology and is particularly familiar with the testicular retrieval of sperm in infertile men. The authors now want to share their experience in the posthumous retrieval of sperm.

It became clear in the early stages of this short study that the technique of retrieval of sperm after death needed much more thought than had been given to it in the past. In modern day reproductive medicine, there is clearly an increasing expectation for IVF and IVF/ICSI to provide a solution to a range of previously uncommon reproductive situations including the use of posthumous sperm to generate embryos and children.

On mentioning the whole concept of posthumous sperm collection to the average Gynaecologist, the response is often that of revulsion. There are declarations concerning the need for the nuclear family, of the possible effect on the child when that child discovers that it was conceived after or even a longtime after the death of its father. Indeed the situation is reminiscent of the response to IVF when Steptoe and Edwards announced the birth of Louise Brown in 1978. At the time the practitioners of IVF were even described as 'murderers' when defective embryos, even those embryos that had no possible potential of the generation of a pregnancy were discarded.

However like it or not, it soon became clear that IVF provided clinicians with a truly amazing advance in the treatment of infertility indicating beyond doubt that the techniques relating to assisted reproduction and *in vitro* fertilization were here to stay. The revulsion that accompanies almost any discussion concerning posthumous sperm retrieval is reminiscent of those days.

However, this technique is starting to be requested by a small number of close relatives of the deceased. The procedure is now also being discussed in the Media and is slowly becoming better known. The situation regarding the Law in Western Australia in relation to posthumous sperm retrieval will one day be sorted out and we hope the cost of the procedure in relation to any Court Order will therefore come down.

In this communication, the main problems concerning this procedure are discussed. It is clear that the clinician must be fully informed of the Law in the jurisdiction in which he or she works: ignorance of the Law may lay the clinician open to prosecution.

We believe that it is important to collect what would seem to be an excess of testicular tissue from these cadavers as one usually has little or no information concerning the deceased's past fertility or even his past paternity and thus the numbers of sperm collected could be reduced. It must also be remembered that the clinician only has one opportunity to collect sperm. If these sperm are in the future to be used to achieve a pregnancy, this may well take more than one cycle of treatment and the number of sperm retrieved must reflect that need. It is also clearly much better to collect more sperm that will be needed in order to optimize the chances of conception.

The role of the needle biopsy needs to be discouraged. Usually only a small number of sperm are obtained from needle aspiration and, in this situation, unlike in life, there is no opportunity for a second biopsy.

Thus, we contend that there is no indication for the use of needle biopsy in posthumous sperm retrieval. A needle biopsy may also not deliver enough sperm for any form of sperm function testing and this should be a further disincentive to use this method of collection.

Overall much more thought must be given to the management of this type of procedure. The posthumous retrieval of sperm should not consist of a quick needle biopsy and the freezing of few sperm. The tissue must be examined carefully and sperm movement assessed. Viability tests may need to be carried out. If the patient is repeatedly charged for the storage of sperm that turn out to be dead, this may initiate unnecessary medico-legal activity. It is also far better to retrieve too many sperm than too few. How much tissue is taken is of little consequence to the cadaver and the risk of taking too little tissue as could well occur in a needle biopsy could prove to be a serious problem.

It is hoped that a discussion of our limited experience of posthumous sperm retrieval and the cryopreservation of those sperm will be of use to clinicians that are involved in this procedure.

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Authors' roles

A.M.J. is the lead author and wrote this manuscript. She also carried out all the sperm collections in these patients. M.Z. performed all the cryopreservation procedures in these patients.

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This work was carried out entirely in the State of Western Australia and thus did not involve the NHS in any way. Any costs that were associated with this study were born by the patient's next of kin and/or his close family. The lead author did not receive any remuneration for any of this work. However the Supreme Court received a substantial sum for their issue of Court Orders as did the Concept Fertility Centre for carrying out the cryopreservation of this tissue. All these costs were handled by the next of kin and/or the close family of the deceased.

Conflict of interest

Neither A.M.J. nor M.Z. has any conflict of interest relating to this study.

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