Bank your future: Insemination and semen cryopreservation

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Abstract

Semen cryopreservation has become an important part of the routine work of most Andrology or Assisted Reproductive laboratories. There are two main reasons for cryopreservation of semen, the one is for the use in donor semen related procedures and the second for the purpose of fertility preservation in males, also known as autoconservation, for many different reasons. These two main areas can further be subdivided into several categories or uses depending on their clinical application.

In this article the emphasis is on storage of semen for autoconservation uses only, with reference to reasons for autoconservation of semen, the effects of cryopreservation on semen parameters and sperm functions and the use(fullness) of cryopreserved semen in practice.

The wide range for autoconservation of semen samples includes *inter alia* the following; conservation of fertility in male cancer patients, treatment of male and/or female subfertility, back-up for ART procedures, fertility preservation before vasectomy or other reasons/procedures and storage of separated X and Y bearing spermatozoa.

It is concluded that semen cryopreservation is an essential tool in ART. IUI with cryopreserved, non-donor, semen is mainly used for cancer patients. Although the request rates for cancer patients and infertility treatment is low, if used pregnancy rates are compatible with other IUI procedures. Despite the low usage rate of stored semen samples the cryopreservation of semen samples in these cases is extremely important given the therapeutic or physiological effect by the knowledge that in worst case scenarios due to the unavailability of fresh semen samples a cryopreserved semen sample is available.

Key words: IUI, semen parameters, autoconservation, cryopreservation, donor semen

Introduction

Semen cryopreservation has become an important part of the routine work of most Andrology or Assisted Reproductive laboratories (Nijs and Ombelet, 2001). There are two main reasons for cryopreservation of semen, the one is for the use in donor semen related procedures and the second for the purpose of fertility preservation in males, also known as autoconservation, for many different reasons (Björndahl *et al.,* 2010). These two main areas can further be subdivided into several categories or uses depending on their clinical application.

For donor cryopreservation semen is obtained from healthy men, or donors, who are presumed to be fertile i.e. capable of impregnation. These donors may be recruited by the clinic or sperm bank and their spermatozoa are then used anonymously, although the contemporary trend is now that the identity of the donor should be made know to the recipients if they so wish. Donor spermatozoa may be used for IUI, IVF or ICSI for a wide range of indications, for instance for an ART procedure of the partner of an infertile man, with no live spermatozoa or elongated spermatids and thus unsuitable for ICSI, where treatment with the male partners semen has failed or treatment became too costly. To prevent transmission of a genetic inherited disorder in heterosexual couples or to prevent foetal haemolytic anaemia in cases of blood group incompatibility or recurrent abortions or for the insemination of single

women or lesbian couples wishing to conceive (Björndahl *et al.,* 2010; WHO, 2010). In this article the emphasis will be on storage of semen for autoconservation uses only, with reference to reasons for autoconservation of semen, the effects of cryopreservation on semen parameters and sperm functions and the use(fullness) of cryopreserved semen in practice.

1 Reasons for autoconservation of semen

The wide range for autoconservation of semen samples includes *inter alia* the following; conservation of fertility in male cancer patients, treatment of male and/or female subfertility, back-up for ART procedures, fertility preservation before vasectomy or other reasons/procedures and storage of separated X and Y bearing spermatozoa.

1.1 Conservation of fertility in male cancer patients

Semen cryopreservation for male cancer patients before chemotherapy, radiotherapy or orchiectomy in cases of cancer or autoimmune diseases, is apart from donor semen cryopreservation one of the most important reasons for autoconservation of semen (Nijs and Ombelet, 2001). This is a very important option for cancer patients were semen can be obtained by masturbation. Other, but less used options available for cancer patients and especially young boys, where it is difficult to obtain semen by masturbation, are testicular tissue banking and spermatogonial stem cell banking with the intention that there will be the necessary progress in research on in vitro maturation of these cells to obtain elongated spermatids or mature spermatozoa.

For patients to undergo cancer therapy with alkylating agents or radiotherapy, the semen must be collected before the patient commences with any therapy in order to decrease the risk of mutagenesis in the spermatozoa. Semen storage before a potential sterilizing procedure often has significant physiological value because of the hope of future paternity.

1.1.1 Effect of cancer treatment on semen parameters and male fertility

It is well known that the kind of cancer and the kind of treatment i.e., chemotherapy with cytotoxic agents or radiotherapy may have different effects on spermatogenesis and eventually on the fertility status of the male. The effect may be permanent or temporally, but it is seldom found that the male will recover to his full fertility potential (Meseguer *et al.,* 2006; Schmidt *et al.,* 2004).

Agarwal and Allamaneni (2005) reported that the mean semen parameters of 205 adolescent cancer patients were severely reduced compared to the semen parameters of a control group. The mean volumes were 1.6 ml and 3.0 ml, respectively. The mean spermatozoa concentrations per ml were 50.6 million and 84.5 million and sperm motility 45.1% and 68.5%, respectively. In a group of 83 males with testicular cancer and a controle group the mean spermatozoa concentrations were 15.0 million and 48.0 million per ml semen, respectively. In a group of males with leukemia the mean sperm concentration per ml semen was 19.5 million compared to 106.0 million of a control group, respectively and the percentage of motile spermatozoa 45.0% and 64.0%, respectively.

Simons *et al*. (2005) found that one year post radiotherapy treatment 16% of men who were initially diagnosed with normozoospermia presented with oligozoospermia and 20% were still azoospermia. However, spermatogenesis in patients who received cancer treatment continued to improve over the next 5 years. Schmidt *et al.* (2007) reported that in a group of males diagnosed with cancer 91.2% of them had fathered a child before treatment, while after treatment only 67.1% were successful to father a child, with a so-called cumulative pregnancy index eventually reaching a pregnancy rate of 85%.

1.1.2 Availability and use of cryopreservation services for cancer patients

In the literature a couple of surveys are available reporting on the availability of semen cryopreservation services and the actual use once the semen samples have been stored. Heath and Stern (2006) reported on a survey done in Australia and New Zealand among paediatric oncology centres. From the 13 questionnaires send out to different centres, 12 centres responded. All 12 centres offered semen cryopreservation but only 9 of the 12 centres offered counselling as a routine procedure. Rofeim and Gilbert (2004) did a survey among oncologists in the USA. Of the responders 91% agreed that semen conservation procedures should be offered but only 27% offered the service sometimes while 10% always offered the service.

1.2 Treatment of male and female subfertility

In men with poor semen samples cryopreservation of their semen with the specific aim to use these cryopreserved samples as an IUI treatment option is not practiced on a wide scale, and only a single article in this regard could be found (Aboulghar *et al.,* 1991). The treatment described by Aboulghar *et al*. (1991) entitled that the subfertile males provided semen samples on a regular basis for cryopreservation. When a sufficient number of samples were stored the best cryopreserved sample of a male was

used for IUI together with a fresh sample. The average pregnancy rates per cycle and the cumulative pregnancy rate in the treatment group was 9.1% (19/73) and 27.1%, respectively. In a control group where only fresh semen samples were used the mean pregnancy rate per cycle was 4.1% and the cumulative pregnancy rate was 13% (9/77). Overall, pooling of multiple abnormal semen samples for AIH has not been proven to be useful.

1.3 Back-up for assisted reproductive procedures and non-cancer fertility preservation

Men may store spermatozoa for treatment of their partner by IUI, IVF or ICSI for many different reasons such as difficulty in collecting semen on the day of the procedure, erectile dysfunction, psychological problems or absence on the day of the ART procedure.

With the introduction of ICSI the possibility for the use of cryopreserved semen became even more widespread as only a few spermatozoa are required for this procedure and the high survival rate in terms of total motile spermatozoa as needed for IUI is not necessary. Men with severe oligozoospermia or intermittent presence of motile spermatozoa in their semen can now use cryopreservation as back-up for ICSI (Bourne *et al.,* 1995). Another widely used application of cryopreservation is the freezing of material obtained by testicular or epididymal biopsies i.e., MESE or TESE. As only a single spermatozoon is needed in ICSI for each oocyte, cryopreservation of any live sperm is worthwhile and all patients, including pubertal adolescents (< 15 years old) boys, as well as from men suffering from spinal cord injuries should be offered the possibility of storage of their spermatozoa (Kamischke *et al.,* 2004).

Fertility preservation for non-cancer reasons are also done for a wide variety of reasons. One of the more frequent applications is before the performance of a vasectomy as insurance against a change in the desire for a child within an existing partner/marital situation or in case of a new partner/marriage commitment where a desire for a new or extended family may occur. In these cases the cryopreservation is performed as insurance for procreation. Other reasons for this kind of fertility insurance is in cases where the husband is performing active duty in a dangerous occupation such as the military forces on condition that it is performed in countries where posthumous procreation is acceptable.

Other reasons may be in cases were a progressive loss of semen quality may be suspected for instance in cases of y-chromosome microdeletions, transient azoospermia or oligozoospermia (Björndahl *et al.,* 2010). Cryopreservation can also be used in cases where it may be suspected that successful treatment of infertility that may not persist, for instance where surgery for genital tract obstruction was performed or after treatment with gonadotrophins for men who are diagnosed with hypothalamo-pituitiary hypogonadism (WHO, 2010). In all these cases and especially in cases where the main reason for cryopreservation is for fertility preservation or male or female infertility treatment, enough specimens should be stored to provide for ten or more insemination cycles to ensure a good chance of a pregnancy.

1.4 Minimising transmission of infectious diseases.

The advent of HIV and the possibility of transmission of the virus via semen or assisted reproductive procedures in the early 1980's required the introduction of a quarantine period for donor semen and further testing of the donor before the stored semen could be used. More recently an alternative method was published where in cases of HIV positive semen a special preparation procedure was employed (Loskutoff *et al.,* 2005). After semen preparation through washing and multiple gradient centrifugation in a special double barrel test tube the resultant spermatozoa from men carrying the HIV diseases can then be cryopreserved for use in ART. Now men with HIV controlled by antiretroviral therapy may store semen samples with undetectable viral load for IUI, IVF or ICSI to attempt conception while reducing the risk of transmission of the HIV to the female partner.

1.5 Separation of X and Y bearing spermatozoa

Semen cryopreservation and IUI has also been used for the purpose of sex selection (Karabinus, 2009). The main reason for this procedure was for the reducing of X-linked genetic disorders or family balancing. Sperm were stained with Hoechst 33342, sorted by flow cytometry, and then used for IUI or cryopreserved for subsequent use for IUI, IVF or ICSI. Fluorescence in situ hybridization (FISH) analysis was used to determined the post-sort enrichment of X- and Y-bearing sperm. The sperm separations performed were in 74.9% of cases for X-sort and in 25.1% for Y-sort. Post-sort purity averaged 87.9% for X-sort and 73.4% for Y-sort. A total of 3629 IUI cycles were performed with a pregnancy rate of 15.6% and a miscarriage rate of 15.7%. Overall X-sort, after IUI, resulted in 92.0% female and Y-sort in 81.5% male babies born. The first outcome from this clinical trail, running from 1994 to 2007, was reported by Fugger *et al*. in 1998.

2 Effects of cryopreservation on semen parameters and sperm functions

It is well known that cryopreservation of semen does have a negative effect on semen parameters and sperm function, especially on sperm motility. On average only 50% of the motile spermatozoa will survive freezing and thawing (Keel and Weber, 1993) with a corresponding reduction in vitality while damage of sperm structure and function also occurs. Alternations in acrosome structure as well as shrinkage of sperm nuclei and cytoplasmic membranes with loss of plasma membrane integrity and the concurrent reduction of intact acrosomes and consequently a reduction in (pro)acrosin activity has been observed (Nijs *et al.,* 2009). Due to the addition of the cryoprotectant there is also a reduction in the sperm concentration. The effect of cryopreservation on semen parameters of cancer patients is in the same order. Menkveld (unpublished data) observed that in 64 patients the mean sperm concentration was reduced from 39.4 ± 28.7 mill/ml (range, 0.1-100.0 mill/ml) before freezing to 18.4 ± 13.5 mill/ml (4.5-40.0 mill/ml) after freezing and thawing. The mean progressive motility (a + b) was reduced from 45.4 ± 13.5% (0.0-60.0%) before freezing to 23.3 ± 12.1% (0.0-40.0%) after freezing and thawing.

With regard to the effect on long term sperm storage on sperm functions and structure Edelstein *et al*. (2008) investigated the effect of storage time on sperm DNA integrity using the TUNEL assay. Semen samples from 14 men were cryopreserved for a short period (1 to 5 years) and from 16 men semen samples were stored for a long term of 9 to 13 years. Edelstein *et al*. (2008) found that in the short term storage group the semen samples contained an average of 31% of spermatozoa with DNA fragmentation compared to the 37% sperm DNA fragmentation of the long term of storage group. The conclusion was that long term storage did not significantly increased sperm DNA fragmentation. Unfortunately they did not presented data on sperm DNA damage before cryopreservation was performed. However, Zribi *et al*. (2010) found that cryopreservation had deleterious effects on sperm DNA by inducing DNA fragmentation and oxidation but could not give an explanation for the underlying mechanism for such damage. Nijs *et al*. (2009) found that cryopreservation had no negative effect on the results of the sperm-hyaluronan binding assay as an average of 68.5% sperm binding was found before freezing and 71.3% after freezing and thawing.

3 Utilisation of cryopreserved semen in practice

Only a small percentage of males who cryopreserved their semen samples will eventually use the stored semen samples. The usage of cryopreserved semen varies between 30% to less than 10% of the stored samples (Simons *et al.,* 2005) but in many centres it may be as low as 5% or less (Chung *et al.,* 2004). In our own experience we found that only 4.7% (3/64) of our patients used their cryopreserved semen samples. In total 3 IUI, 2 ICSI and 1 GIFT procedures were performed (Menkveld, unpublished data).

3.1 Use of cryopreserved semen/sperm in cancer patients

In early reports cryopreservation of semen for cancer patients with the intention for the performance of artificial insemination was thought to be pointless if motility was <40% and sperm concentration was < 20 million spermatozoa per mL, due to unacceptable low chances for conception. Even if the semen quality before cryopreservation adhered to the above mentioned criteria pregnancy rates were very poor. Therefore, as a result even today many oncologists consider semen cryopreservation for cancer patients as insufficient and not worthwhile to perform. With the introduction of IVF and especially ICSI this situation changed drastically as only a few motile spermatozoa are needed for these procedures (Tournaye *et al.,* 2004).

Ragni *et al*. (2003) reported that over a 15 year period, involving 776 men with malignant diseases, 5.2% (36/686) of these men used their cryopreserved samples for ART with cumulative pregnancy rates after 4, 8 and 12 years of 4.5%, 8.7% and 11.8%, respectively. Magelssen *et al.,* (2005) reported their experience with the use of cryopreserved semen from cancer patients over a twenty year period. They reported that 7% (29/422) of men used their cryopreserved semen samples at least once or sometimes more than once for assisted reproductive procedure in order to try to achieve fatherhood. Of these men 55.1% (16/29) achieved a pregnancy, while 17% (67/393) of men achieved a pregnancy without use of their cryopreserved semen samples. In a group of men who did not cryopreserve their semen before cancer treatment, 21% (205/966) achieved a pregnancy. Hourvitz *et al*. (2008) reported on the results of 118 couples who underwent 169 IVF cycles using cryopreserved pre-cancer treatment sperm. The observed clinical pregnancy rate was 56.8% per retrieval with a total of 96 pregnancies, resulting in 126 children born and 11 spontaneous abortions. Semen samples from males presenting with prostate cancer showed the poorest semen parameters before cryopreservation and also achieved the lowest pregnancy rates. Van Casteren *et al*. (2008) reported on the use of 749 cryopreserved semen samples from 557 men over an average follow-up period of 7 years. Over this period, 42 patients requested the

use of their cryopreserved semen samples. In a group of 37 patients where the results were available, 32 IVF cycles were performed, 53 ICSI cycles, 9 cryo-embryo transfers and 7 IUI's, resulting in 8, 16, 2 and 1 pregnancies, indicating that pregnancy rates for IVF and ICSI's were significantly higher than those for IUI. Over the follow-up period 7.5% of the patients used their cryopreserved semen resulting in a live birth of 49%. Crha *et al*. (2009) performed semen cryopreservation for 619 cancer patients over an 11 year period from 1995 to 2006. In this period of time 32 (5.2%) of the patients sought infertility treatment and cryopreserved semen samples were used for 28 couples who underwent a total of 44 ICSI cycles, resulting in 13 pregnancies.

Time of storage

Semen stored under appropriate conditions will show no deterioration of sperm quality with time and children have been born following fertilization using semen stored for over 28 years (Clark *et al.,* 2006; Feldschuh *et al.,* 2005). In one couple two pregnancies resulted after IUI treatments performed with cryopreserved semen samples stored for 21 and 28 years, respectively (Feldschuch *et al.,* 2005).

Pregnancy rates with stored semen

Best pregnancy rates with artificial insemination performed with cryopreserved donor semen are no different from those achieved with fresh semen and pregnancy rates range from 15% to 25% per month, or cycle, of treatment. However, pregnancy rates of 7-12% per month are more common. Rates are often related to post-thaw sperm quality, timing of insemination and particularly influenced by recipient factors such as age of the women and tubal or uterine disorders (Le Lannou and Lansac, 1993).

Evidence suggests that there is no increase in abnormal pregnancy outcomes after the use of cryopreserved semen for IUI. The rates for spontaneous abortion of 13%, major birth defects of about 1% and the sex ration of 51:49, males:females, are similar to those of spontaneous pregnancies (Lansac and Royere, 2001).

Conclusion

Semen cryopreservation is an essential tool in ART. IUI with cryopreserved, non-donor, semen is mainly used for cancer patients. Although the request rate is low, if used pregnancy rates are compatible with other IUI procedures. The storage of semen from cancer patients should be offered, after sufficient consultations, to all cancer patients before cancer treatment because it is a mental booster and if not offered legal action can be taken against the treating institution. If semen is stored for the treatment of a subfertile couple in cases were the male presents with poor semen parameters, the frozen samples are mainly used for IVF and especially ICSI procedures. The question is very often asked if it is worthwhile to do outo(cryo)conservation of semen due to the fact that the frozen samples are not used on a great scale. The answer is that the therapeutic or physiological effect given by the knowledge that in case of worst scenario cryopreserved semen is available is a sufficient reason for performing this procedure.

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