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SPERM DONATION PROGRAMME AT THE CENTRE OF ASSISTED REPRODUCTION IN BRNO: RESULTS FROM 1995–2005

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Abstract

Sperm donation has been an integral part of the assisted reproduction programme at the Department of Gynaecology and Obstetrics of Masaryk University and the Faculty Hospital in Brno since 1995. In spite of great progress in the assisted reproduction possibilities the usage of donor sperm has its own irreplaceable place, because there will always exist couples for whom it is a unique solution to beget their own offspring.

During the 10-year period, after fulfilling the specific seminal, microbiological, serological, and genetic requirements, there were sorted out only 65 donors out of 280 donor candidates. More than 4500 samples were cryopreserved and 2800 samples were used for intrauterine inseminations and oocyte in vitro fertilisation.

Key words

Donor, Semen, Cryopreservation, Sperm, Insemination

Abbreviations used

ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilisation

INTRODUCTION

When clinicians started to be aware of the fact that azoospermia or very severe oligospermia could not be improved by medical treatment, the idea of creating sperm banks arose. Using donor sperm became an integral part of assisted reproduction. Before the introduction of micromanipulation techniques, mainly the oocyte intracytoplasmic sperm injection (ICSI) (*1*), (*2*), it was the only possibility of reaching one's own child in cases with low sperm count, sporadic sperm, poor motility, immotility, or in a combination of these factors. In spite of markedly reduced usage of donor sperm because of the possibility of obtaining fertilisable sperm from the epididymis (Microepididymal Sperm Aspiration) or the testis (Testicular Sperm Extraction) as well in some cases there remains an irreplaceable place for the donor sperm.

The donor sperm is used to treat couples with azoospermia, severe male factor infertility, significant sperm or seminal abnormalities, in families carrying genetic diseases which may be transmitted by the husband's spermatozoa, in cases of Rh incompatibility, previous failure to fertilise, and in some countries with HIV seropositive males (*3*).

Efficient cryopreservation and storage of semen samples is an important condition for the sperm bank establishment. The storage duration is not limited. Cryopreservation is known to cause some changes in the sperm morphology, including damage to mitochondria, the acrosome, and the sperm tail. The sperm motility is particularly sensitive, and it is generally accepted that it can be reduced to one half after a cryopreservation/thawing procedure (*4,5,6*). Due to this fact, it is necessary to choose potential donors with an emphasis on this sperm parameter.

Before becoming donors, all candidates are interviewed and screened by our rules. Sperm is used only after six months of storage, when negative serology is confirmed. The donation is anonymous and free of charge in the Czech Republic. Only the expenses connected with donation are covered for the donor.

The sperm donor programme has a ten years´ tradition at our workplace, and our sperm bank belongs to the largest in our republic. Some other centres of assisted reproduction are interested in our donor semen. Sperm is used for intrauterine insemination (IUI), classical in vitro fertilisation of oocytes, or for ICSI.

MATERIALS AND METHODS

From the beginning of 1995 to April 2005, 280 prospective donors had been registered in our Centre of Assisted Reproduction. The possibility of becoming a donor was heard of from friends – other donors, from leaflet campaigns in blood transfusion places and residential halls, and from articles published in magazines.

Admission Requiements

Donor candidates are recruited from 18- to 35-year-old volunteers, of minimally secondary school education with good health status and absence of genetic abnormalities. During the selection, a personal and family medical and genetic history form is completed with the aim to discard potentially inheritable disorders. A questionnaire focused on phenotypic characteristics (hair colour, hair texture, eye colour, height, weight, blood type) and character and interests is also filled out. Before becoming donors, all of them have to pass the semen testing and microbiological, serological and genetic examinations. They are fully informed about anonymity and further details, and provide a signed consent for the use of their gametes.

Requisite Investigations

1. Semen samples examination

Semen samples, collected by masturbation, are examined within 1 hour after ejaculation into a sterile container (abstinence for 2 to 3 days). After liquefaction a semen analysis is carried out – the semen volume, sperm concentration, motility percentage, and sperm morphology are evaluated according to the WHO Manual (*7*) in a Makler chamber. The minimal semen parameters recommended for the donors are mentioned in *Table 1.*

Table 1
Minimal semen parameters

Volume > 2 ml
Sperm motility > 60% moving actively
Sperm concentration > $50x10^6$ sperm/ml
Sperm morphology normal range
Cryosurvival > 50% of initial motility

2. Microbiological sperm examination

The basic bacteriological examination for anaerobic and aerobic microorganisms and the examination for Ureaplasma urealyticum and Mycoplasma hominis presence are performed.

3. Serological examination

Prospective donors are screened for sexually transmitted and infectious diseases by: serological test for syphilis, serum testing for hepatitis B surface antigen, hepatitis C antibody (HCV), serum testing for HIV-1 and HIV-2, and testing for ALT. Blood type and Rh factor are determined. Each sample is cryopreserved for a half-year period and can be used until after a repeated negative test confirmation. If any of the tests mentioned above is positive, the donor must be rejected and offered appropriate counselling and treatment.

4. Genetic evaluation

A full family and genetic history is taken, and caryotyping is carried out. The cystic fibrosis carrier status is eliminated.

Informed consent

A donor signs the informed consent form, in which he declares that he is a man without a history of sex with men, and has not injected drugs for non-medical reasons. He knows that he must announce any changes in his state of health, e.g. transmitted sexual diseases, without delay.

Anonymity protection

Sperm donation is anonymous in the Czech Republic. The donor samples are marked by a numerical code. When used, they are transliterated once more. In this way not only anonymity between the donor and the recipient but also between the doctor and the laboratory staff is guaranteed.

The use of donor sperm may be carried out only in a married couple after the informed consent has been signed. The couple can complete a questionnaire respecting the donor characteristics. Phenotype, blood type, and Rh factor are taken into account.

Donation and its limits

The internal rules for donation and its limits have been defined. The donor should come to the semen taking minimally once a week until 20 – 30 sperm samples are cryopreserved or until 8 – 10 children from his sperm were born. This period is called donor cycle.

Sperm cryopreservation and storage

For the semen sample cryopreservation Richardson's medium prepared by ourselves was used from 1995 to 2001. Its base was buffer, glucose, fructose, and sodium citrate. Glycerol was used as a cryoprotectant. Glycine and egg yolk served as a protein source. Since 2000 we have started to perform cryopreservation with commercially produced media from Medi-Cult (Denmark) or IVF Science and/or Vitrolife (Sweden).

Semen is mixed with cryomedium and portioned into plastic tubes. The samples are frozen by one of two methods - either by means of a Planer Kryo F10 programmable equipment by the standard freezing curve or in nitrogen vapour only.

The samples are stored in Dewar vessels in liquid nitrogen. The nitrogen volume is inspected and supplied at the vessels once a week.

Sperm thawing and preparation for insemination

After withdrawing from the Dewar vessel the cryotubes are left at room temperature. The thawed semen is prepared by a swim-up procedure. During rinsing with a preparation medium the cryoprotectant is removed. The total number of motile spermatozoa in the final preparation is calculated.

RESULTS

The interest to become a donor was shown by 280 candidates 18–5 years old (23.6±3.9) during a decade. On the basis of substandard semen parameters 187 candidates (66.8 %) were refused. Out of 93 candidates invited for microbiological, serological and genetic examinations, 18 did not come. Lack of interest after having learnt the result of the spermiogram was the probable reason. Seven men out of 75, who had passed through a genetic evaluation, presented inconvenient findings. The causes of their rejection were: positive biochemical screening for a metabolic inherited disease (3x), mutation dF508 heterozygote carrier (2x), objectionable karyotype (1x), family genetic load (1x). After passing all the requisite investigations, 65 men (i.e. 23.2 %) became donors out of 280 previous candidates.

Donor cycle termination

Out of 65 selected donors only 33 men finished the whole donor cycle successfully. According to the semen taking frequency the donor cycle ranged from 1 to 3 years. The others stopped donating for various reasons:

1. deterioration in the semen quality – in 6 cases decline of concentration and motility, in 7 cases a low quality after the cryopreservation/thawing procedure;
2. seven men stopped at their own request;
3. 12 donors stopped for unknown reason;
4. six donors reached 10 born babies.

The number of samples

Since the sperm bank establishment, 4680 semen samples have been cryopreserved. 2819 samples were withdrawn and used up to date. 1233 samples were used for IUI and 916 samples for IVF. 670 samples were not suitable for insemination. Further 4 centres of assisted reproduction from the Czech Republic have been interested in our donors´ semen. They have withdrawn 405 samples since the year 2000.

DISCUSSION

The recruitment and selection of semen donors is a problematic procedure (*8*). Only relatively few donors are recruited compared with those screened. More than one half of the interested persons (67.0 %) was rejected because of poor sperm quality in the first examination. They were men without any previous experience in this field. This examination may signalise some potential problems to be solved by them in future. Nearly 20 % of men with good sperm parameters did not come any more after the first examination. It can be speculated that it was only a good chance for them to find out their own sperm parameters free of charge, or they changed their minds for various reasons. As no seropositive candidate was met, some could probably belong to those mentioned above. Positive microbiological detection was very rare and antibiotic cure was sufficient for its elimination. Inconvenient genetic screening occurred only in 10 % of the candidates.

The correct functioning of a semen donation programme requires, besides well-established cryopreservation, an exhaustive control of both clinical and legal aspects. The most important aspect is to avoid any transmission of infectious and genetic diseases to the gamete recipients and their progeny (*9*). The second is the control of the offspring obtained from the donors in order not to exceed the maximum recommended number of newborns. At our Centre we have established a limit of 8 – 10 babies from one donor, while, for instance, Dutch workplaces permit as much as 25 offspring from one donor (*10*).

It is important to select candidates who donate their gametes from a low risk population for both infectious and genetic diseases and after an adequate selection, to maintain a strict control of blood analyses and microbiological cultures.

On the basis of our observations we may conclude that the application of donated sperm is an extremely safe option for infertile patients where the use of their own gametes is not possible or where a continuous assisted reproduction treatment failed, if all directives are strictly observed. Finally, the patients must be informed about the fact that the total absence of risk by using the donated sperm cannot be completely assured.

For the reasons mentioned above and management intensiveness of the whole donor programme it is more convenient for some private centres to buy cryopreserved samples from our sperm bank.

CONCLUSIONS

During the 10 years' duration of the sperm donor programme at our Centre, 65 (23.2 %) suitable donors were selected out of 280 candidates after a strict selection and fulfilment of the specific seminal, microbiological, serological and genetic requirements. More than 4500 samples were cryopreserved. About 2000 women from our or other Czech centres wished to use donor sperm for intrauterine inseminations and oocyte in vitro fertilisation. The sperm donation programme has its

irreplaceable place, even if significant progress in assisted reproduction techniques has been achieved, because there will always exist couples for whom it is the only solution to beget their own offspring.

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PROGRAM DÁRCOVSTVÍ SPERMATU V CENTRU ASISTOVANÉ REPRODUKCE V BRNĚ: VÝSLEDKY 1995 – 2005

Souhrn

Dárcovství spermatu je součástí programu asistované reprodukce Gynekologicko–porodnické kliniky FN a LF MU v Brně od roku 1995. Program dárcovství spermatu má i přes velké pokroky v možnostech a technikách asistované reprodukce své nezastupitelné místo a stále budou existovat páry, pro které je to jediné řešení ke zplození potomka. Za 10 let trvání bylo po splnění všech předepsaných kritérií a spermiologickém, mikrobiologickém, serologickém a genetickém vyšetření vybráno 65 (23,2 %) vhodných dárců z 280 zájemců o darování. Zamraženo bylo přes 4500 dávek a spotřebováno přes 2800 dávek pro intrauterinní inseminace a in vitro fertilizaci oocytů.

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