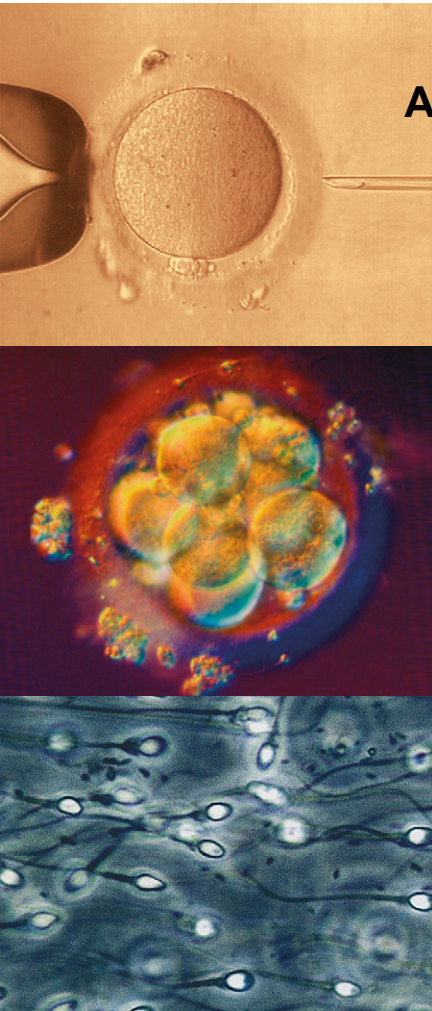


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Assisted Reproduction Using Cryopreserved Sperm – a Mini Review

K. H. Baumann, A. Weidner, M. Kalff-Suske, K. Bock

This mini review summarizes a short lecture given at the “Crash Course Cryopreservation of Spermatozoa”, on May 20, 2006, in Marburg, Germany. Diagnostic and therapeutic measures and the possibilities of the use of cryopreserved sperm in ART are discussed. *J Reproduktionsmed Endokrinol* 2007; 4 (2): 97–100.

Key words: sperm cryopreservation, assisted reproduction technology

Interdisciplinary cooperation characterizes the approaches in diagnostics and therapy of the infertile couple. The prevalence of infertility among couples of reproductive age is estimated at 15 % [1]. In approximately 25 %, various causes of fertility problems are present in both partners, and in nearly 35 %, low sperm count or quality are assumed the sole cause of fertility problems. These etiological figures clearly underline the necessity of interdisciplinary concepts in fertility diagnostics and treatment to include several medical and associated disciplines (Tab. 1). Joined together, clinic, laboratory, and research will eventually optimize current treatment options. The term „Assisted Reproductive Technologies“ (ART) summarizes, with respect to the female reproductive situation, all methods beyond cycle monitoring in the presence or absence of ovarian stimulation. Measures of ART include intrauterine insemination (IUI) and in vitro fertilization (IVF). Techniques of assisted fertilization like intracytoplasmic sperm injection (ICSI) and other measures using and handling sperm and eggs are also part of ART.

Diagnostics of Female Infertility

A careful and elaborate medical and life style history will point to possible reasons for female infertility, while at the same time leading to an adequate patient-physician relationship. Endocrine and non-endocrine disorders might become obvious already upon reported symptoms (e.g. polycystic ovarian syndrome). Clinical and technical investigations as listed in Table 2 should be chosen when indicated. Invasive diagnostic procedures such as hysteroscopy or laparoscopy should be employed if the

Table 1. Disciplines involved in the diagnostics and therapy of the infertile couple

• Gynecology	• Psychiatry
• Urology	• Neurology
• Andrology	• Sexual therapy
• Human genetics	• Clinical chemistry
• Surgery	• Medical microbiology
• Internal medicine	• Molecular biology
• Psychology	• Further disciplines as indicated

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From the University Hospital of Gießen and Marburg, Location Marburg, Germany

Correspondence: Klaus H. Baumann, MD, Department of Gynecology, Gyn. Endocrinology and Oncology, University Hospital of Gießen and Marburg, Location Marburg, D-35043 Marburg, Baldingerstraße, Germany; e-mail: baumannk@med.uni-marburg.de

selection of further treatment options will be based on these results.

Diagnostics of Male Infertility

Assessing male fertility also comprises non-invasive as well as invasive methods. A medical and andrological history (e.g. mumps, maldescensus testis, trauma, ejaculation disorders, malformation) may indicate male fertility disorders and will lead to specific further testing.

The assessment of sperm count and quality is a basic and important prerequisite for further decisions. Following three to five days (some centers recommend two to seven days) of abstinence, ejaculated semen should be evaluated. The WHO criteria of normal sperm count and quality are listed in Table 3. Semen analysis is highly sensitive and subfertility values can be rapidly recognized. However, semen analysis lacks specificity. In case of an abnormal result, semen analysis is recommended to be repeated twice in a three-month interval.

Clinical investigation of the male patient should focus on the whole body: hair distribution, fat distribution, height and body composition and configuration, also assessing

Table 2. Diagnostic measures in female infertility

- Medical and gynecological history
- Clinical and gynecological investigation
- Basal temperature
- Monitoring of menstrual cycle
- Endocrine laboratory diagnostics (β -HCG, LH, FSH, estradiol, progesterone, 17 β -hydroxyprogesterone, testosterone and free testosterone, androstenedione, SHBG, DHEAS, prolactin, TSH [additional values and stimulation or suppression tests as indicated])
- Ultrasonography (abdominal and vaginal)
- Hysterosalpingosonography
- Hysteroscopy
- Laparoscopy
- Further diagnostic measures as indicated

Table 3. WHO criteria for normal semen quantity and quality

- Volume: 2.0 ml or more
- Liquefaction time: within 60 min
- Sperm number: 40 million spermatozoa per ejaculate or more
- Sperm concentration: 20 million spermatozoa or more per ml
- Motility: 50 % or more WHO grades a and b or 25 % or more WHO grade a
- Morphology: 15 % or more of normal morphology
- Vitality: 50 % or more live
- pH 7.2 or higher
- Peroxidase positive cells: less than $1 \times 10^6/\text{ml}$

Table 4. Diagnostic measures in male infertility

- Medical and andrological history
- Clinical and andrological investigation
- Ultrasonography (abdominal, scrotal, transrectal)
- Duplex sonography
- Semen analysis, CASA
- MAR (mixed antiglobulin reaction) test
- Sperm function tests
- Endocrine laboratory diagnostics (testosterone, FSH, LH, prolactin, inhibin B, estradiol, SHBG, stimulation tests if indicated)
- Further diagnostic measures as indicated

possible female sex proportions. Penis, scrotum and testis should be carefully assessed. Measuring testis volume by orchidometer or ultrasonography provides further information.

Additional technical, laboratory, and molecular test methods are listed in Table 4.

In case of azoospermia, invasive diagnostics are required. Spermatozoa can be retrieved from the epididymis or testis. Multilocular biopsy is usually performed to increase the chances for testicular spermatozoa detection. Already at this point it is important to offer the opportunity for testis biopsy cryopreservation. Cryopreserved biopsies can be used for spermatozoa retrieval in case of later assisted reproduction therapy.

Current Concepts in ART

Currently, ART include among others: IUI, IVF, ICSI as mentioned above, as well as GIFT (gamete intrafallopian transfer), ZIFT (zygote intrafallopian transfer), ET (intrauterine embryo transfer), PESA (percutaneous epididymal sperm aspiration), TESA (testicular sperm aspiration), TESE (testicular sperm extraction from a testicular biopsy), SUZI (sub-zonal insemination), PGD (pre-implantation genetic diagnosis). Cryopreservation of reproductive cells and tissues is of increasing importance [2–4].

Indication for Sperm Cryopreservation

Patients needing to maintain their reproductive reserve or those undergoing ART therapy represent the main target groups for sperm cryopreservation.

In case of intrauterine insemination or in vitro fertilization using donor sperm, prior cryopreservation is required with quarantine criteria being the most obvious reason.

Even though the use of freshly obtained semen is recommended, cryobanking of partner/husband sperm might be useful prior to IUI or IVF. Storage and pooling of more semen samples is required in case of reduced semen quality, revealing subfertility or an inability to obtain sufficient and qualitatively adequate semen upon therapeutic demand in ART.

Planned surgical treatment of males due to a malignant or non-malignant disease might also call for sperm cryopreservation. Any surgical procedure directly affecting the male reproductive organs, connected neurons or blood supply of the male reproductive organs or their function can lead to the recommendation of prior sperm cryopreservation.

Following successful surgical intervention, e.g. resulting in the restoration of male reproductive capacity, the cryopreservation of sperm might be taken into consideration because of possible secondary therapeutic failure or recurrence of infertility.

Treatment for malignancies might require sperm cryopreservation. Two reasons account for this recommendation: iatrogenic loss of reproductive capacity, and – to some extent – increased risk of mutagenic harm to reproductive cells due to chemotherapy or irradiation. It has been shown that among men only 20 to 50 % regain fertility and sufficient spermatogenesis following chemotherapy [5].

Treatment for non-malignant diseases may also indicate sperm cryopreservation. Diseases requiring immunosuppressive or cytotoxic therapy [6] as well as diabetes or vascular disorders may account for male fertility problems.

The demand for sperm cryopreservation due to an increase of hazardous life situations will rise. Globalisation – not only with respect to military issues and dangerous professional occupations – requires worldwide mobility and engagement, possibly leading to yet unknown threats on health and well being, also with respect to reproductive capacity.

Sperm cryopreservation and postmortem use in ART or retrieval of spermatozoa from a deceased man is morally and legally highly controversial and thus subject to varying regulations in different countries. In the authors' country, the legal situation does not allow postmortem sperm retrieval or use.

Precautions and Methods

Clinical, microbiological, and laboratory testing of patients and samples has to exclude the risk of pathogen transfer prior to spermatozoa cryopreservation. Diverse protocols are available for sperm cryopreservation. The techniques are described elsewhere in this issue.

Success in ART Using Cryopreserved Sperm

Despite optimized cryopreservation and thawing procedures of sperm, the motility of frozen-thawed sperm is negatively influenced. Comparing prefreezing to post-thaw values, the percentage of motile spermatozoa is reduced by 25–35 %. The quality of motility and the morphology of cells was shown not to be significantly altered by freezing and thawing [7, 8]. Still, sperm motility represents a surrogate marker for sperm fertilization capacity.

Lower pregnancy rates upon usage of frozen-thawed sperm in IUI were reported [Sherman, 1973]. The probability of achieving a pregnancy using fresh spermatozoa in IUI was three times that of frozen-thawed semen. The success rate measured in resulting pregnancies after IUI was 18.9 % for fresh semen [9]. Decreased sperm parameters following freezing-thawing were confirmed by others [10].

For ICSI, the differences in pregnancy rates between using fresh or cryopreserved sperm are not significant

[11–13]. Moreover, the origin of cryopreserved spermatozoa, either ejaculated or retrieved by invasive methods, (MESA, TESE) results in similar success rates in ICSI [14]. None of the cited studies demonstrated a significant disadvantage of frozen-thawed spermatozoa in ICSI. Using cryopreserved spermatozoa obtained by TESE, a non-significant decrease of pregnancy rates, compared to freshly obtained and used spermatozoa, was reported [15].

IVF using cryopreserved sperm is also an option [16], however decreased sperm motility might interfere with the outcome and ICSI should be recommended.

In a Spanish follow-up study, 186 men opted for sperm cryopreservation prior to oncological treatment. Thirty ICSI cycles were performed from frozen samples achieving clinical pregnancies in 50 % [17]. This is in accordance with findings from Agarwal et al [18], who reported a 37 % pregnancy rate following ICSI cycles with cryopreserved sperms, showing it to be more successful than performing IVF or IUI with banked cryopreserved sperm.

In case of any invasive andrologic diagnostic measure like testis biopsy, remaining tissue or cells are to be cryopreserved upon request of the patient. It is a physician's obligation to proactively inform the patient about these methods, alternatives and consequences. Nevertheless, in obstructive and non-obstructive azoospermia, the recovery of testicular spermatozoa via TESE can be repeated successfully [19]. Thus, sperm cryopreservation and repeated invasive measures are complementing methods. Sperm cryopreservation to some extent avoids invasiveness without decreasing success rates.

Each cooperating center for reproductive medicine thus should provide the skills and technical facilities to perform cryopreservation and storage of spermatozoa, making therapy of infertility again an interdisciplinary approach.

The coordination of ART, in most cases consisting of IUI, IVF and ICSI, often lies with the gynecologist. During monitoring and treatment of women, either in non-stimulated or stimulated cycles, the timing of ovulation and the coordination of assisted reproduction procedures will be scheduled by gynecologists. In case of invasive spermatozoa retrieval, a close cooperative interaction with andrologists and urologists is important, in case of cryopreserved sperm close cooperation with the service providing laboratory is of profound importance. Logistic procedures need to be planned in advance.

Outlook

Risk of sample infection, cross-contamination, or infection transfer is actively reduced. Instruments for risk reduction include patient screening prior to semen collection and storage for sexually transmitted diseases including HIV, and hepatitis B and C. Quarantine of samples for six months and rescreening of donors in case of use of donor semen are effective tools to avoid transfer of infections.

ICSI and the use of cryopreserved sperm seem to be associated with a higher rate of genetic disorders in offsprings [20], even though contradicting data have been published [4]. The resistance of sperm from infertile men to

the mutagenic potential of sperm cryopreservation is reduced compared to sperm from healthy fertile men [7]. Additional technical efforts are required to decrease the risk of DNA damage or to detect and exclude mutated sperm [7, 21].

There is no evidence that cryopreservation of sperm results in transfer of malignant cells from male cancer patients to women or their offspring [2].

There is a problem, however, connected with the transfer of genetically caused male infertility [22, 23]. The idiopathic sertoli-cell-only syndrome, related to the deletion of the complete AZFa sequence, will be transferred via ART [22]. This shows one distinct syndrome – among others – that is not only relevant in respect to fertility, thus highlighting the yet not completely resolved problem of disease transfer related to genetic alterations in men. Further development of reliable, specific, sensitive and affordable screening methods for genetic mutations is required.

Sperm cryopreservation methods have to be optimized in respect to decreasing the damage on DNA and biological sperm function. Presumably, increasing success rates of ART using freshly obtained spermatozoa will be paralleled by rising success rates in obtaining pregnancies by using frozen-thawed spermatozoa.

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