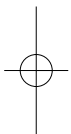
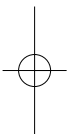
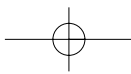


# ASSISTED HUMAN REPRODUCTIVE TECHNOLOGIES AND HIV

Consulting Committee on Assisted  
Human Reproductive Technologies  
in Catalonia



This document has been approved in the Fourth Meeting  
of the Consulting Committee on Assisted Human Reproductive Technologies  
in Catalonia, held on May 31, 2002 in the Generalitat de Catalunya  
Health and Social Security and Department headquarters.



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## 1. INTRODUCTION

Catalonia is, for different reasons, pioneer in Spain in the advances in assisted reproductive technologies and is considered an international reference for the introduction of novel technologies in this field. This leadership is also echoed in the application of these procedures in HIV-carrying individuals, considering the particularly high prevalence of the infection in couples of childbearing age. In this manner, and as in other countries, the Consulting Committee on Assisted Reproductive Technologies in Catalonia has decided to make a statement that will contribute to meeting user needs and questions and that will be ethically sound and compatible with professionals' good practice.

This document addresses those issues related to the desire of giving birth to a child by couples in which the female partner, the male partner or both partners are HIV carriers. The changing views and rationales taken toward this infection, which previously recommended that these couples should not procreate, are now, in light of recent advances, being reconsidered. However, this does not mean that the measures that have so far proven effective in the control of the disease will be diminished.

Even though every disease is subject to an individual analysis, we believe that the recommendations set forth in this document are, in general, applicable to infections other than HIV.

## 2. EPIDEMIOLOGY

According to estimates made by the "Centre d'Estudis Epidemiològics sobre la Sida de Catalunya (CEESCAT)", the number of individuals infected with HIV in Catalonia at the end of 2000 was approximately 28,572 (20,483-36,565); that is, 0.46% of the total Catalan population, or 7 of every 1,000 people between 15 and 64 years of age.

	Estimated population	No. of infected individuals
Women (15-49 years old)	1,604,741	4,005
Men (15-64 years old)	2,047,080	11,237
Number of pediatric AIDS cases in Catalonia: 213 (0.68%) of 13,136 reported cases between 1981 and 2000.		

The CEESCAT data indicate that there could be approximately 4,000 women and 11,237 men of fertile age infected by the HIV virus. These data have been calculated using an index population, and are an approximation of the global prevalence rate of the infection within a reference population. Despite the limitations of these data, there is a trend toward a decrease in the number of infected individuals in Catalonia, from 0.53% to 0.46%.

In order to confirm these data, a monitoring system has been developed for new HIV infection diagnostic techniques, which, as of January 1, 2001, through physicians' confidentiality agreements, will allow greater accuracy of the data.

### 3. VERTICAL TRANSMISSION

In our society, if no preventive measures are taken, the rate of HIV vertical transmission ranges between 15% and 20%. Spain has the highest number of reported pediatric AIDS cases in Western Europe. This significant number of HIV-1-infected children can be explained by the high prevalence of the infection in women of fertile age. According to the National AIDS Cases Registry, the first diagnosed case of vertically transmitted AIDS in a pediatric patient dates back to 1984. Until June 2000, a total of 837 cases of vertically transmitted AIDS had been reported. Madrid, Catalonia and Andalucia had the highest number of cases.

The highest incidence occurred in 1988, with a total of 90 infected infants. This number remained stable until 1995, when the introduction of antiretroviral agents (ZDV) for the prophylaxis of vertical transmission caused a marked decrease in the number of cases (12 cases in 2000), which suggests that the trend for the coming years may be toward a decrease. In developed countries, the obstacles to eliminating vertical transmission are the mothers who do not consult a physician until the late stages of pregnancy or at the time of delivery, the majority of whom are addicted to drugs.

Thanks to the application of effective preventive methods, especially with the use of highly active antiretroviral therapy (HAART), the current rate of vertical transmission ranges between 1% and 2%. For example, one may note that in the Hospital Clinic of Barcelona, where there are 25 to 30 births per year among infected women, there has not been a case of vertical transmission reported since 1998.

Despite the fact that there have been reported maternal or fetal complications due to the use of antiretroviral agents, these have been sporadic. However, the long-term effects of these agents are still unknown.

#### 4. PROGNOSIS

The prognosis for these patients has changed. Previously, HIV was considered a fatal infection, and practically all patients developed serious manifestations of the disease, with clinical complications of AIDS occurring within a time frame of approximately 10 years. The survival rate after the initial occurrence of these complications—the most frequent being pneumonia caused by *Pneumocystis carinii*—used to be 1 year. Currently, highly active antiretroviral therapy using combination agents (HAART), in countries where this therapy is available, has delayed and even avoided the progression of the disease toward AIDS, increasing the life expectancy of infected patients and significantly decreasing the number of deaths. AIDS may currently be considered a chronic disease, and it is expected that much of the infected population will be free of inherent complications. For this reason, some individu-

als who have the disease, many of whom are asymptomatic and have a favorable life expectancy, consider the possibility of having a child. The immediate consequence of this situation is the need for positive reproductive counseling, which will not recommend, as before, the avoidance of pregnancy in those couples in which one or both members are seropositive.

The current low rate of mother-to-child transmission, as well as the remarkable improvement in the disease's prognosis, have given these couples the desire to have children. In light of this, it is imperative that the health care professionals involved are able to offer these individuals answers that will adequately meet their needs.

## **5. FERTILITY AND HIV**

### *Female fertility and HIV*

A decrease in fertility was described years ago in women infected with HIV, but the numerous factors involved in this area make it difficult to study the relation between HIV and fertility. Some studies suggest that HIV may have a negative effect on fertility, in both symptomatic and asymptomatic women. Furthermore, other studies signal an increase in the rate of spontaneous abortions.

### *Male fertility and HIV*

Important changes have been reported in the seminogram (oligo, asteno and teratospermia), the causes of which are not fully known. On the contrary, other studies do not find expressive differences between HIV-positive patients and those not infected.

## **6. CURRENT STATUS OF REPRODUCTION IN HIV-POSITIVE MEN**

The most frequent case is the demand for reproduction in semidiscordant couples in which the male partner is the HIV carrier. It is estimat-

ed that the probability of being infected through sexual intercourse without the use of protection depends on several factors: viral load of the infected individual, presence of sexually transmitted diseases and immune factors, among others. The chances of becoming infected through sexual intercourse with no protection ranges between 0.08% and 0.2%. The use of condoms has traditionally been recommended to avoid the risk of transmission of the noninfected partner. In general, the recommendation to partners wanting to have a child is artificial insemination with sperm donation, even though this implies that the partner will not be the biological father.

It has been demonstrated that semen can transmit HIV. Although the virus's presence in the spermatozoa seems unlikely because of its lack of receptors, this remains a controversial issue. The quantity of virus present in semen depends on many factors. On one hand, it is not always possible to detect the virus in all infected individuals; on the other hand, the presence of free viral particles has been found in semen or integrated in the cellular portion of the ejaculated liquid (proviral form) in HIV-infected individuals receiving HAART therapy, with undetectable levels of viral charge in plasma. This confirms the role of the male genital tract reservoir for HIV. Although these individuals may have an undetectable viral load, they also have the capacity of sexually transmitting the virus and are therefore forced to continue the use of condoms during sexual intercourse, which makes fertilization impossible.

Semprini (1992) published data on 12 pregnancies of serodiscordant couples achieved through artificial insemination after mobile spermatozoa were separated from the rest of the ejaculatory fluid. In this small group, none of the mothers seroconverted and no newborn was infected.

Mandelbrot (1997), considering the low infection risk in unprotected intercourse, did not find necessary the need for artificial insemination with previous semen wash in serodiscordant couples. He also recommended having sexual intercourse without using protection during the periovulatory phase. A total of 104 pregnancies were obtained in 92 couples, although four of the women seroconverted (4.5%).

Furthermore, Semprini (1997) published data on more than 1,000 artificial inseminations in 350 serodiscordant couples, obtaining 200 pregnancies without any cases of seroconversion to HIV in women or infection to the newborn. These favorable results indicate the need for protection in sexual intercourse, as the risk of infection, although low, did exist.

More recently, various groups in Barcelona have used the same methodology, attaining similar results.

	ART	Couples	Cycles	Preg- nancies	Deliv- eries	Live births	Ongoing preg- nancies	Vertical trans- mission	Horizontal trans- mission
Total	AI	581	1122	252	187	215	35	0	0
no. of cases	IVF	148	198	92	45	62	28	0	0
Data up to March 30, 2002									
ART: assisted reproduction technology; AI: artificial insemination; IVF: <i>in vitro</i> fertilization.									

Following the semen wash, the presence of HIV is determined through polymerase chain reaction (PCR) in order to confirm the effectiveness of the wash. The results obtained by the different research teams that, up to now, have applied this technique (with no cases of seroconversion) suggest that the semen wash followed by a negative PCR confirmation is a safe and reproducible technique, both for artificial insemination and for *in vitro* fertilization. Although the possibility of seroconversion in women cannot be completely ruled out, the risk seems to be minimal. On the other hand, the main objective of this technique is to reduce the risk when the desire of pregnancy is great.

## 7. CURRENT STATUS OF REPRODUCTION IN HIV-POSITIVE WOMEN

The desire of pregnancy is no different in seropositive women than in those who are not seropositive. As stated above, the risk of mother-to-child transmission in a woman with controlled disease is, at most,

between 1% and 2%. The antiretroviral therapy required by infected women for the control of the disease is, in general, enough to avoid vertical HIV transmission. Therapy agents may cause problems, such as hyperglycemia (protease inhibitors) or mitochondrial toxicity (nucleosides), that occur as neuropathies, myopathies and lactic acidosis, among others, with the risk of serious implications to the mother or fetus. Fortunately, these problems are not frequent and are generally reversible. On the other hand, although the real effects of the use of antiretroviral therapy, including during the first trimester of pregnancy, are not yet known, it seems unlikely that this therapy may be associated with malformations.

As with any other disease associated with perinatal risk, preconception counseling is essential. HIV-infected women must plan their pregnancy and must be well informed of the possible complications that might develop. Women with immune and viral stability and receiving effective antiretroviral therapy may choose to undergo pregnancy with a minimal risk. In these cases, if the male partner is not infected, self-insemination or emptying of the sperm-filled condom into the vagina may be recommended.

Problems occur when the couple does not reach a spontaneous pregnancy or requires assisted reproductive technologies. To date, this circumstance has been almost always a formal contraindication to any type of assistance. In this case, the medical staff must address and answer a series of questions:

- Is there risk of perinatal HIV infection when trying to become pregnant?
- Is the risk of transmitting HIV infection to health care professionals acceptable?
- Is the risk of contaminating other laboratory samples acceptable?

The last two questions are easy to answer: in an appropriately equipped laboratory, the risk of HIV contamination of health care professionals and of biological samples is minimal. As to the first question, the right to procreate is an individual's right. Health care personnel must know how to offer individuals the appropriate assistance in reaching this goal

and may not discriminate under any circumstances. Other patients with serious diseases (diabetes, antiphospholipid antibody syndrome) or with hereditary conditions that may be associated with poor perinatal results (extreme prematurity, mental or growth retardation, etc.), may be counseled in a routine manner in human reproduction centers.

In our opinion, the existence of HIV infection in a woman cannot be a contraindication to her assistance, as her exclusion would not be ethical. In any case, as with any other serious disease, individual counseling would be essential.

The currently favorable quod vitam prognosis for women, the low risk of vertical transmission and of therapy-related complications, along with the appropriate laboratory conditions, support the fact that HIV-positive female patients should receive the same counseling as women with other serious diseases. Decisions must be made on a one-to-one basis for each couple, but must never be based solely on HIV infection.

## **8. LEGAL ASPECTS OF THE APPLICATION OF ASSISTED REPRODUCTION TECHNOLOGIES IN HIV-INFECTED INDIVIDUALS**

In reference to the legal aspects surrounding this issue, it is important to distinguish two different situations:

1. Techniques involving donor gametes or preembryos. In this case, the RD412/1996 seems to prohibit the use of genetic material that may yield positive in HIV screening tests. Article 4.2 states that “in the case where any of the tests results are positive, for exclusion effects, this result will be notified to the National Registry with the purpose of safeguarding the appropriateness of sanitary information and guidelines.” This is one of the many directives that tend to avoid the transmission of diseases that, in this case, affect donors, so that seropositive individuals that test seropositive may not be gamete or preembryo donors.

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2. In the case that an assisted reproduction technique utilizes genetic material from a couple in which one of the partners is seropositive. In this case, the solution provided in number 1 above is not applicable for the following reasons:
- a. The aims of prohibiting donation are not met, and it would be the same as prohibiting reproduction in couples suffering other types of disease; this would translate into eugenism, which is currently legally prohibited.
  - b. If a semen wash that avoids HIV transmission in a safe manner is technically possible, one must consider that article 1.3 of law 35/1988 estimates the use of assisted reproduction technologies “also in the prevention and treatment of diseases of genetic or hereditary origin, given the appropriate diagnostic and therapeutic guarantees, and that are strictly indicated.” Furthermore, even though HIV infection is not a disease of the type described in article 1.3, it may be similar in terms of the applicability of the law, given that the disease may be transmitted in the forms described in the legal disposition.
  - c. The same law, 35/1988, allows preembryonic interventions if and when the objectives of such interventions are the detection of hereditary diseases with a therapeutic intent, if possible, and when they result from the need to offer the “well being of the *nasciturus* and its favorable development.” Since these interventions are authorized in preembryos (article 12) and in embryos and fetuses (article 13), one concludes that semen wash treatment may be perfectly used in this type of situation.

In conclusion, the use of assisted reproduction technologies in HIV-infected couples is possible in the attempt to apply the most adequate technology to avoid viral transmission, if and when all requirements established in law 35/1988 and in article 1.3 are met. Otherwise, it would be impossible to authorize gamete donations by HIV-infected individuals, as a consequence of the prohibitions set in RD 412/1996.

## 9. WHICH ATTITUDE SHOULD ONE TAKE?

As seen, Catalonia is a high prevalence area for HIV. Therefore, the request for assisted reproduction technologies is not unusual and

obliges professionals to address the issue. There are two attitudes to this problem:

1. Exclude these couples, or the infected female partner, from the possibility of assisted reproduction technologies as a means of fully avoiding vertical HIV transmission, or because there is not an appropriately equipped laboratory for the handling of contaminated samples.
2. Include these couples in assisted reproduction technologies.

One must bear in mind that everyone has the right to reproduce, and that HIV infection, in either of the two partners, must not be an absolute contraindication to pregnancy. Furthermore, the current evolutionary trend of this disease does not justify any kind of discrimination toward these couples.

In this manner, it is interesting to recall that in the last months, several international authorities have changed their recommendations on the matter, facilitating the application of these technologies in their countries (2001):

#### France

*Arrête du 10 mai 2001 modifiant l'arrête du 12 janvier 1999 relatif aux règles de bonnes pratiques cliniques et biologiques en assistance médicale à la procréation. J.O. Numéro 112 du 15 mai 2001, page 7735.*

*Avis n 56 du Comité Consultatif National d'Ethique pour les sciences de la vie et de la santé "Problèmes éthiques posés par le désir d'enfant chez des couples ou l'homme est séropositif et la femme séronégative". 10 de février de 2001.*

*Avis n 69 du Comité Consultatif National d'Ethique pour les sciences de la vie et de la santé "L'assistance médicale à la procréation chez les couples présentant un risque de transmission virale. Reflexions sur les responsabilités". 8 de novembre de 2001.*

#### USA

*Human immunodeficiency virus and infertility treatment. Ethics Committee of the American Society for Reproductive Medicine Birmingham, Alabama. USA, October 25, 2001.*

## Germany

*Diagnostics and Treatment of HIV-Discordant Couples Who Wish to Have Children. M.M. Weigel<sup>1</sup>, H. Kremer<sup>2</sup>, U. Sonnenberg-Schwan<sup>3</sup>, J. Götz<sup>4</sup>, L. Gürtler<sup>5</sup>, H.W. Doerr<sup>6</sup>, N.H. Brockmeyer<sup>7</sup>. European Journal of Medical Research, 2001.*

## Spain

Publications by various scientific societies: GESIDA, SEIMC, Asociación Española de Pediatría (AEP), Plan Nacional sobre el Sida y Sociedad Española de Ginecología y Obstetricia (SEGO), and various authors: J.A. Iribarren, J.T. Rams, L. Guerra, O. Coll, M.I. de José, P. Diumenge, C. Fortuny, P. Miralles, F. Parres, J.M. Penya, C. Rodrigo i R. Vidal. Enfermedades Infecciosas y Microbiología Clínica. Vol. 19, número 7, agosto-septiembre 2001 (Publicación oficial de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica).

*Criterios para la utilización de los recursos del Sistema Nacional de Salud Español en técnicas de reproducción humana asistida. Grupo de Interés de Centros de Reproducción Humana Asistida del Sistema Nacional de Salud. Revista Iberoamericana de Fertilidad, vol. 19, n 11, enero-febrero 2002.*

We believe that the existence of HIV infection in women and men does not have to represent a discriminatory situation to the access to assisted reproduction technologies. In any case, the complexity of this subject requires fully dedicated and specialized teams and attention to the constant changes and advances that develop both in human reproduction as well as in HIV infection.

According to the rationales presented above, teams must perform the following procedures:

- Appropriately equipped laboratory (vertical flow bells and independent freezing containers), a well defined and established sample reception and handling circuit, and well-defined standard operating

procedures to safeguard laboratory personnel as well as noncontaminated samples.

- Professionals must be experienced in this field and an established circuit of infection control of these patients should be implemented.
- Preconceptional counseling, as with any other couple with fertility problems, especially in cases in which one of the partners is HIV-discordant; counseling on safe sex is needed, and the use of condoms must be reinforced to avoid infecting the noninfected partner.

By following the procedures outlined below, couples should have access to the same assisted reproduction technologies as noninfected couples, and no different tests will be required, except for those needed for particular aspects of HIV infection.

## **10. PRELIMINARY STUDY OF THE COUPLE**

- In female and male partner:
  - Cervical or urethral cultures
  - Serological evaluation (Chlamydiae, gonorrhoea, syphilis, hepatitis)
- In infected female and male partner:
  - HIV-specific tests (CD4, viral load)
  - Infectious disease specialist report confirming the partner's optimal condition, and in the case that the female partner is infected, that the risk of vertical transmission is minimal
- In non-infected female or male partner:
  - HIV test
- Counseling of both partners who wish to undergo treatment

In all cases, the responsible team will perform thorough preconception counseling in which all possible problems are outlined, including the risk of infection of the noninfected partner, HIV vertical transmission and problems related to the use of antiretroviral therapy during pregnancy.

In the case of pregnancy in an infected woman, she will be addressed to an experienced reference center. Informed consent forms will be filled out for each procedure.

## **11. SEMIDISCORDANT COUPLES (HIV-POSITIVE MALE, HIV-NEGATIVE FEMALE)**

Acceptance criteria:

- Male partner's clinical condition:
  - Appropriate medical follow-up by infectious disease specialist (compliance to scheduled visits and indicated therapy)
  - Absence of opportunistic infections or serious complications associated with HIV infection (displasia, serious CNS disorders, etc.)
  - Strict criteria on plasmatic viral load will not be required; in fact, couples that could most benefit from the semen wash would be those in which the male partner has a higher viral load
- Consistent condom use in the last 4 months.

Criteria to evaluate exclusion:

- Active drug use (heroin, cocaine, etc.)
- No use of condoms in a systematic manner
- Serious complication of the infection during evaluation/treatment

It is possible to use two techniques: artificial insemination and *in vitro* fertilization. For either, it is necessary to:

- 1) Couple's informed consent.
- 2) Perform specific semen wash and PCR evaluation of the sample. Results must be negative both for RNA and DNA.
- 3) PCR, HIV or antibody and p24 antigen (women) tests with negative results 15 days prior to either procedure. These tests will be repeated whether pregnancy is obtained or not. In the case that pregnancy is obtained, a quarterly HIV serological determination will be performed.

## 11.1 ARTIFICIAL INSEMINATION

The procedure will always be directed toward artificial insemination, ensuring that the semen sample is optimal for this technique and that the female partner does not suffer any concomitant condition or disease that specifically calls for *in vitro* fertilization.

### *Treatment protocol*

In order to improve control of the cycle and maximize the probability of success, the insemination cycle will be performed through an ovarian stimulation treatment with gonadotropins or, in exceptional cases, with clomifen citrate or spontaneous cycle. General artificial insemination guidelines will be observed in the treatment protocol, cycle monitoring and insemination procedure and technique.

## 11.2. *IN VITRO* FERTILIZATION

*In vitro* fertilization will be recommended, following the same indications used for any infertile couple. This technique consists of the transference of embryos obtained after oocyte insemination with spermatozoa previously treated through semen wash and confirmed HIV-negative. Prior to initiating the *in vitro* fertilization cycle, a semen sample previously screened for HIV will be frozen. This sample will be used in the fertilization procedure.

The treatment protocol, cycle monitoring, oocyte retrieval and embryo transfer will be performed following the general *in vitro* fertilization guidelines. In order to minimize oocyte exposure to spermatozoa and therefore to minimize the possibility of infection, the recommended technique is intracytoplasmic sperm injection (ICSI). Special attention will be given to ovarian stimulation treatments and to the number of transferred embryos in both techniques in order to avoid multiple pregnancies.

## **12. SERODISCORDANT COUPLE (HIV-NEGATIVE MALE, HIV-POSITIVE FEMALE)**

When pregnancy is not reached, the most appropriate assisted reproduction technology will be recommended, after instructing the couple on condom emptying procedures (condoms without spermicide agents) and on self-insemination, or in the presence of a primary indication.

Inclusion criteria:

- Stable clinical condition, CD4>200 cells/cm<sup>3</sup>, low or undetectable viral load and a thorough medical evaluation, to minimize vertical transmission
- Appropriate medical follow-up by infectious disease specialist (compliance to scheduled visits and indicated therapy)
- Absence of opportunistic infections or serious complications associated with HIV infection (active neoplasia, serious CNS disorders, etc.)
- Patient commitment to correctly following the indicated treatment, and following the current recommendations on HIV infection during pregnancy and on the prevention of mother-to-child transmission

Exclusion criteria:

- Active drug use (heroin, cocaine, etc.)
- Substitute treatment with morphine agents (methadone)
- Occurrence of a severe complication during evaluation or treatment

In the case that the male partner is also HIV-positive, the criteria to be followed in evaluating his inclusion or exclusion in an assisted reproduction technology program will be the same as those indicated above in “Preliminary study of the couple.”

### **12.1. ARTIFICIAL INSEMINATION**

This will be performed as outlined in the prior section (in the case of semidiscordant couples). A semen wash and viral screening from the obtained semen sample will not be necessary if the male partner is HIV-negative.

## **12.2. *IN VITRO* FERTILIZATION**

Indications and methodology for this procedure are the same as those for noninfected couples. Medical and nonmedical personnel involved in the process will use the same measures recommended for any type of intervention in an HIV-infected individual. Handling of contaminated samples will be done following standard laboratory procedures detailed in Annex 1.

## **13. CONCLUSIONS**

The presence of HIV infection in women or men must not be a discriminatory condition to accessing assisted reproduction technologies, given the need for this type of procedure for reaching pregnancy or for avoiding viral transmission. One must consider the fact that everyone has the right to reproduce. In either case, given the complexity of this matter, dedicated teams are needed, as well as particular attention to the constant changes and advances that develop in both human reproduction and in HIV infection. Dedicated personnel must count on appropriately equipped laboratories with well-defined standard operating procedures to warrant their own protection as well as for noncontaminated samples.

As in any other case of assistance to couples with infertility problems, it is necessary to perform a preconception counseling, particularly when the couple is HIV-discordant. In this case, adequate guidance on safe sex will be provided, and the use of the condom will be reinforced in order to avoid infection of the noninfected partner. Current legislation allows for a favorable interpretation of the preventive use of assisted reproduction technologies to couples in these conditions.

Finally, it is important to realize that the use of the procedures that follow the guidelines described in this document turns the desire of couples, in which one of the partners is HIV-infected, to give birth into a feasible option, as it also reduces the risk of vertical transmission. We believe that public health system should take into consideration the application of such technologies given the fact that they may but to decrease HIV infection.

## **Annex 1**

### **HANDLING HIV-CARRIER PATIENT SAMPLES**

#### **A. Visits**

General precautions must be taken when collecting blood samples (gloves), vaginal ultrasounds with a double condom, disposable vaginal speculum and other surgical equipment (Pozzi tweezers, dilators or hysterometers), which must be heat sterilized.

#### **B. Laboratory/surgery room**

Steps in which special precaution must be taken when dealing with seropositive patients, so as to avoid personnel infection or horizontal HIV transmission.

##### *Overall precautions*

A predetermined area will be dedicated to this type of sample (benches, hoods...) to be handled by appropriately equipped professionals.

All disposable equipment (pipettes, test tubes, speculums, insemination or transference canula) will be stored in a container available at each of these areas in order to avoid circulation of used materials in the laboratory or surgery room. This material will be considered contaminated and will be destroyed using a controlled waste disposal procedure. Needles and glass material will be avoided. No material will be reused that cannot be properly sterilized.

Following all the procedures, material that is not disposable will be properly sterilized at each corresponding work area (stereomicroscope glass, Unopipette adapter and test tube racks). This will be done jointly and in accordance with personal protection measures. A Hexanios-type detergent (25 ml/5 l) will be used for 15 minutes, followed by rinse with water and finally with sterile, distilled water.

Personal protection measures will apply to all personnel directly involved in these procedures. Follicular fluid and semen will be collected with a mask, protective goggles and gloves.

Spilling of contaminated samples will be particularly avoided, but in the case that this should occur, appropriate measures will be immediately taken for the hygiene and decontamination of the affected area.

### **B.1. Semen wash procedure for HIV-infected men (intrauterine insemination and *in vitro* fertilization)**

A semen wash will be performed on HIV-infected men following the technique that proves most efficacious, so as to obtain a fraction of mobile spermatozooids free of seminal plasma and of infected cells, as, for example, that described by Semprini.

HIV (RNA and DNA) presence will be determined in all washed samples for all cases, following the appropriate laboratory procedures. *In vitro* insemination/fertilization will not be performed unless this result is obtained and a negative result confirmed.

### **B2. Sample treatment procedure for *in vitro* fertilization in HIV-infected women**

Medical and nonmedical personnel who participate in the process will adopt the recommended measures in any type of intervention on an HIV-infected or hepatitis B or C infected individual. The follicular suction reflux deposits will be heat sterilized.

A minimum of four washes will be done on recovered oocytes so as to eliminate most blood cells. A small cotton filter will be placed on the tip of the automatic pipettes (it is convenient to always work with tips of these types).

The hood and its instruments (stereomicroscope and 37 plaque) will be covered with filter paper, and incubator trays will be covered in aluminum paper. This way, any spilled drop may be easily picked up, avoiding contamination. The hood and incubator must be used exclusively for this type of patient.

Semen sample processing is the same as that described for intrauterine insemination.

One will attempt to perform all possible procedures in the hood. In the case that a step must be performed outside the hood, this will be done in the designated work area for this type of sample, and all adequate measures will be taken in order to avoid team and instrument contamination.

The storage of the obtained samples (spermatozoa, embryos) will be done in separate freezing tanks.

**Annex II**  
**INFORMED CONSENT FORM MODELS**

**Informed Consent for the performance of artificial insemination or *in vitro* fertilization in semidiscordant couples (HIV-positive male and HIV-negative female)**

Mr. .... ID no. ....  
 Mrs. .... ID no. ....

The undersigned constitute a couple in which the male partner presents positive HIV antibodies and the female is negative. We express our desire to reproduce, and for this reason, wish to be included in the assisted reproduction or *in vitro* fertilization program, with treated semen for HIV serodiscordant couples.

We are aware that achieving pregnancy in our case, by means of sexual intercourse, represents a risk of HIV-1 transmission. We are informed of the developed semen sample treatment techniques for laboratories, followed by intrauterine insemination or *in vitro* fertilization, which are able to reduce the presence of HIV-1 in semen and, henceforth, considerably minimize the risk of transmission.

Considering prior comments, and the fact that the reliability of the applied techniques (sample handling procedures and HIV detection technique in semen) is not 100% safe, and having been informed of current alternatives to obtain pregnancy (donor semen artificial insemination), we freely accept the responsibility of minimum risk of contamination that might derive from the use of the above mentioned technique.

In order to eliminate the possibility of seroconversion, the patient agrees to perform, whether if pregnancy is obtained or not, a PCR-HIV determination in blood or, if necessary, of antigen p24. Furthermore, in the case of pregnancy, the patient agrees to perform an HIV antibody determination every 3 months until delivery and, should it be necessary, to follow a recommended medical treatment. In the same manner, the couple commits to perform indicated medical treatments and analyses on the newborn.

We hereby express our desire to be submitted to an artificial insemination or *in vitro* fertilization with previously laboratory-treated and HIV-screened semen.

Read, signed and accepted  
 by both members of the couple  
 (names and signatures)  
 Place and date

Physician  
 (name and signature)

**Informed consent for the performance of artificial insemination of in vitro fertilization in serodiscordant couples (HIV-negative male and HIV-positive female)**

Mr. .... ID no. ....  
 Mrs. .... ID no. ....

The undersigned constitute a couple in which the woman presents positive HIV antibodies and the man is negative.

We express our desire to reproduce and, for this reason, wish to be included in the assisted reproduction or *in vitro* fertilization program.

We are aware that achieving pregnancy in our case, by means of sexual intercourse, represents a risk of HIV-1 transmission for the couple as well as for the newborn.

We understand that assisted reproduction technologies will only be performed in the case that the viral charge of the woman is undetectable and that the CD4 lymphocyte number is at least 200/mm<sup>3</sup>.

In the case of pregnancy, I agree to follow the recommended treatments, medical controls, laboratory tests and check-ups during pregnancy and at delivery.

In the same manner, the couple commits to perform indicated medical treatments and analyses on the newborn.

We have been informed that the effects of antiretroviral therapy on the fetus are unknown and that undergoing adequate treatment does not ensure that the future infant will be born without HIV antibodies. Nonetheless, we know that following a correct treatment reduces this risk significantly.

We hereby express our desire to be submitted to an artificial insemination or *in vitro* fertilization with previously laboratory-treated and HIV-screened semen.

Read, signed and accepted  
 by both members of the couple  
 (names and signatures)  
 Place and date

Physician  
 (name and signature)

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